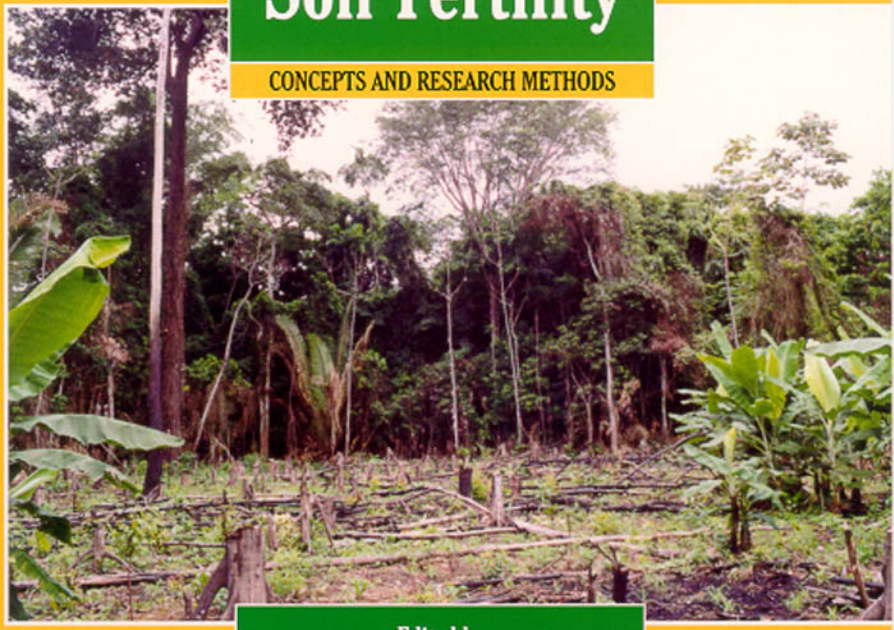
Trees, Crops and Soil Fertility

CONCEPTS AND RESEARCH METHODS



Edited by
G. Schroth and F.L. Sinclair

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VISIT…



Trees, Crops and Soil Fertility
Concepts and Research Methods

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Foreword

Soil science benefits from the availability of a wide array of practicable methods. There are a number of very useful compendia in which sets of these methods are collected together to provide easy reference for the intending practitioner. The need for yet another handbook might therefore be questioned. This book, however, fulfils several needs that are not met in previous volumes.

First and foremost it is unique in its format and purpose – being not a compendium of protocols but a reasoned discussion of the value and utility of different methods. Major advances in science are dependent on, and sometimes even driven by, the availability of suitable methods by which key questions or hypotheses may be answered. Progress may be said to be a product of the match between developments in concept and those in technique. The structure and content of this book are designed to review the ways in which current thinking in terms of the major problems of soil fertility can be methodologically attacked. As such the book should be useful not only in helping to solve problems but also in provoking new questions.

The book is written within a particular context – soil fertility development under agroforestry. At first this may seem very specific and thus limited in appeal and application. But over the last decade or so agroforestry research has been one of the most influential in developing new insights into soil biology and fertility and thus provides a very suitable framework for review of progress. Furthermore, the influence of trees on soil is profound and of significance beyond agroforestry systems, so the book is likely to be of interest in the wider spheres of agriculture, forestry and ecological sciences.

The book covers a spectrum of soil methods broader than most soil science handbooks, combining the techniques of soil chemistry and physics with a wide scope of biological approaches and aspects of social science. This is reflective of the ways in which soil research has moved beyond its strict reductionist paradigm to embrace a more holistic and ecosystematic approach.

The book comes at an appropriate time to review the progress of both concept and method in the slow march towards an integrated approach to land management. It should not only serve the purpose of directing enquiring researchers towards the useful approaches but also act as a stimulus for the next wave of soil fertility research.

Mike Swift
TSBF, Nairobi, Kenya

Editors' Note

This book was produced as part of a project of the International Union of Forest Research Organizations (IUFRO) on the development of manuals for research in agroforestry in association with the World Agroforestry Centre (ICRAF) and the Tropical Agricultural Research and Higher Education Centre (CATIE). Mike Swift, former Director of the Tropical Soil Biology and Fertility Programme (TSBF), has endorsed the book on behalf of the TSBF programme within the International Centre for Tropical Agriculture (CIAT).

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Chapter 1
Impacts of Trees on the Fertility of Agricultural Soils

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1.1 Trees and the Development of Agriculture

Trees are a natural component of most tropical landscapes, with the exception of very dry areas, tropical alpine ecosystems and a few other regions with extreme soil or climatic conditions which do not permit the establishment of woody perennial plants. In the humid tropics, trees are the dominant components of the natural vegetation, the tropical rain forests. With decreasing total rainfall and increasing length of the dry season, these are replaced by drier forest types and then by savannas. Although the density of trees decreases and their crown cover becomes more open with increasing aridity of the climate, trees are present and play an important role in natural ecosystems from the perhumid tropics almost to the fringe of the desert.

Humans have had a pronounced influence on tropical ecosystems and their tree cover for a long time and continue to do so now. Fire and the axe, or its modern equivalent the chainsaw, are the main tools employed by farmers, planters and pastoralists to reduce tree cover and increase the availability of light and soil resources for their crops and pastures. Either tropical forests and savannas have been transformed by shifting cultivators into patchworks of swidden fields and different stages of fallow regrowth, or sedentary farmers have converted natural vegetation more permanently into crop fields, pastures, tree crop plantations or human settlements. Some of these areas may eventually be abandoned and revert to secondary forest or savanna vegetation, unless woody regrowth is prevented by recurrent fires, as in the *Imperata* grasslands of South-east Asia (Garrity *et al.*, 1997).

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Although the rise in human populations has caused pronounced reductions in tree cover, trees remain an important element of most human-dominated landscapes throughout the tropics. Trees provide a wide range of important products and services that people in the tropics want and need. These range from firewood and construction materials, through many different fruits, nuts, medicines, gums, resins and fodder, to services such as shade, wind protection and aesthetic and spiritual value (Scherr, 1995). Tree cover also provides important habitat for both the conservation of wildlife and the utilization of many non-timber forest products that people harvest, including a number of valuable plants, fungi and game animals. Less visibly, but no less importantly, trees play a crucial role in maintaining and regenerating soil fertility through the action of their roots and litter.

Tropical farmers are conscious of these different functions of trees and have protected, planted, selected and domesticated trees for thousands of years. Shifting cultivation systems in tropical forests depend on the regeneration of soil fertility under the forest fallow, which also provides game and a variety of products for collection. Shifting cultivators in Indonesia and other tropical regions have introduced tree crops such as rubber into their swiddens and have created secondary forests enriched with valuable trees (Gouyon *et al.*, 1993). Farmers in West African savannas maintain valuable trees, which also resist periodical fires, in and around their fields, giving rise to a distinct, park-like landscape (Boffa, 1999). Planters of tree crops such as cocoa, coffee and tea maintain or establish shade trees to reduce pest and disease pressures and nutrient requirements of their crops and protect them from climatic extremes (Beer *et al.*, 1998). Since many tree species can fulfil these functions, they may choose species that fix atmospheric nitrogen and produce large quantities of nutrient-rich litter and prunings, or valuable timber or fruit tree species, or any combination of these. Pastoralists value trees for the high nutritional value of the fodder from their leaves and fruits, which in seasonally dry pastures are still available when the grasses have dried out (Cajas-Giron and Sinclair, 2001). They may also prefer to plant trees as living fence posts rather than to replace timber posts every few years when they have been consumed by termites and fungi. Farmers in eastern and southern Africa have recently started to use short-term, planted fallows with legume trees to regenerate the fertility of their soils more rapidly than with natural fallows and to substitute for mineral nitrogen fertilizer, which is often too expensive for them to purchase (Kwesiga *et al.*, 1999). These fallows may also produce valuable animal fodder.

All these and many more practices that involve growing trees in some form of spatial or temporal combination with crops or pastures are known as agroforestry. They have drawn substantial interest from scientists and development agencies during recent decades, recognizing the fact that

trees can play an important role in income generation and food and fuel security for resource-poor rural households, while underpinning the sustainability of their farming systems (Cooper *et al.*, 1996). This recent upsurge in interest in agroforestry may give the impression that it is a new science. This is not the case, as interactions between tree crops and shade trees, for example, have been studied by agronomists for more than 100 years and concepts of nutrient cycling, which are still relevant, were developed early on (see, for example, Lock (1888) on shade trees for coffee in Sri Lanka). Agroforestry is thus a relatively recent word for a much older science and a very old practice. However, efforts to make better use of trees in rural development had (and sometimes still have) to overcome initial antipathy between agriculture and forestry, institutionalized in government departments, research centres and educational establishments, which have led to an arbitrary separation of research and administration of forests and farming.

What is agroforestry?

There are numerous definitions of agroforestry which stress different aspects of and expectations about the integration of trees in farming landscapes (see, for example, Huxley, 1999). Following the predominant definition over the past two decades,

> agroforestry is a set of land use practices that involve the deliberate combination of woody perennials including trees, shrubs, palms and bamboos, with agricultural crops and/or animals on the same land management unit in some form of spatial arrangement or temporal sequence such that there are significant ecological and economic interactions among the woody and non-woody components.
>
> (Sinclair, 1999)

Traditionally, the focus of agroforestry research has been on interactions between trees and other components of a system, such as crops, soil and climatic factors, on the scale of an individual field or a small section of a landscape. This is gradually changing as better understanding of small-scale processes enables researchers to scale up their results, and as the functions of tree cover manifest at landscape, regional and global scales, as a result of larger-scale patterns and processes, become the focus of research interest. These functions include water and nutrient cycling on the catchment scale, carbon dynamics in the soil–plant–atmosphere system and biodiversity. Also, agroforestry practices do not exist in isolation, but interact with other land uses across landscapes. A farmer maintaining a forest garden or shaded tree crop plantation may also have swidden or irrigated rice fields and pasture which occur together within

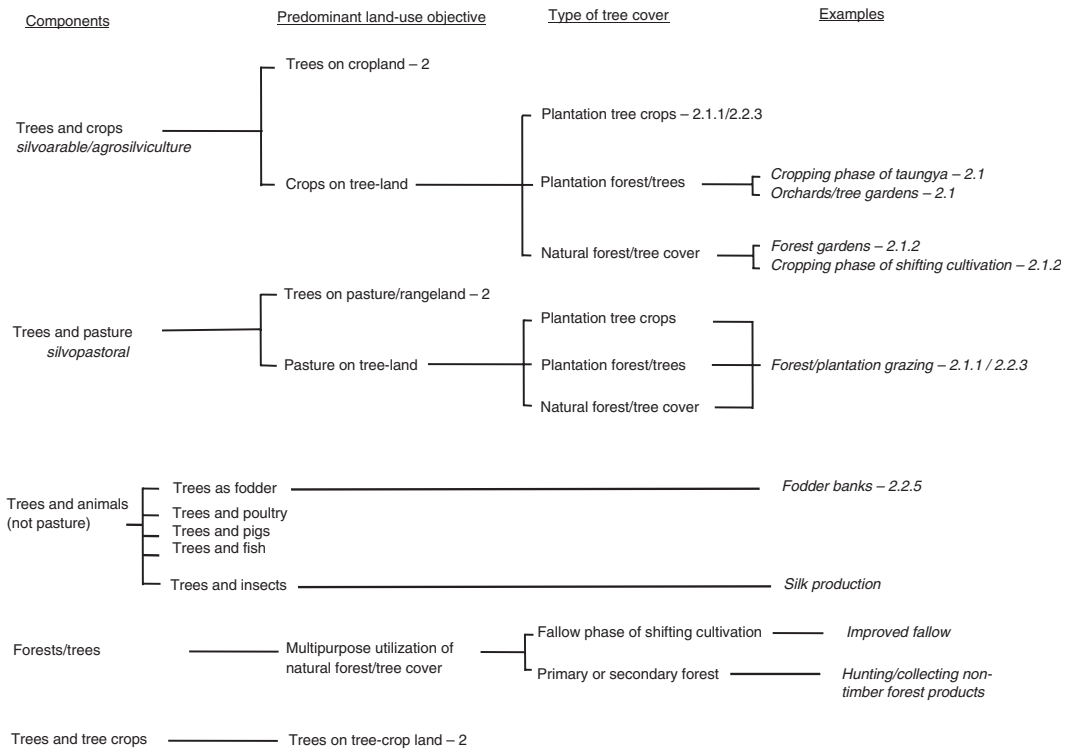


Fig. 1.1. Primary classification of agroforestry practices based on components, predominant land-use objective and the type of tree cover. Italics indicate examples of agroforestry practices, numbers refer to the place in Fig. 1.2 where classification continues (adapted from Sinclair, 1999).

the same landscape and influence the characteristics of this landscape. This wider focus of agroforestry research is reflected in a scale-neutral definition of agroforestry as simply 'where trees and agriculture interact' (Sinclair, 1999).

Within this wider view of agroforestry, the landscape scale is emerging as a critical unit of analysis (Sinclair, 2001). This is the scale at which ecological processes such as the presence and dispersal of fauna and flora, water and nutrient flows, microclimate, and pest and disease dynamics are significantly influenced by trees. In many fragmented landscapes trees on farms, including those shading tree crops or that occur as remnants in crop fields or pastures or in riparian corridors, provide key elements of the tree cover that determine landscape characteristics. Strategic placing of trees in the landscape may prevent, enhance or direct flows of soil, water, nutrients, fire and organisms across landscapes (van Noordwijk *et al.*, 1999). Where there is a mosaic of agriculture and forest, interactions between these land uses determine such important environmental functions as the water yield of catchments and landscape-scale biodiversity

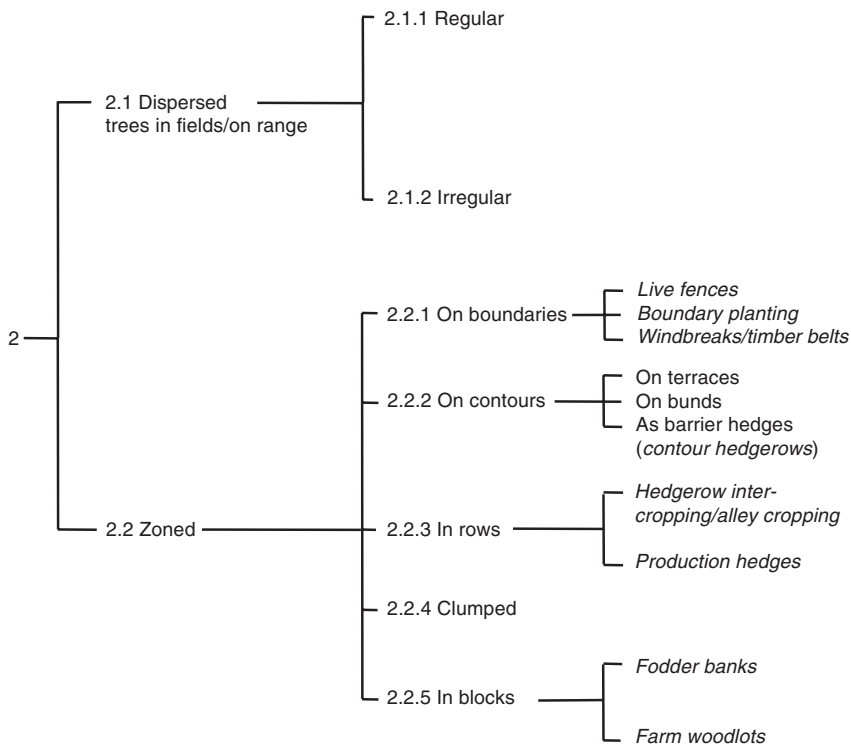


Fig. 1.2. Secondary classification of agroforestry practices based on density and arrangment of the tree component (adapted from Sinclair, 1999).

(Guindon, 1996; Bruijnzeel, 1997). In the future, efforts to understand and develop the role of trees on farms will increasingly focus on landscapes.

In order to facilitate communication, the many ways in which farmers use trees within their farming systems have been classified into several major types of practice, on the basis of the components that are involved, the type of land on which they occur and the type of tree cover involved (Fig. 1.1). These major types of practice can be further classified in terms of the density and arrangement of the tree component (Fig. 1.2). This results in defining groups of practices which share important ecological and managerial characteristics. Lists and descriptions of common agroforestry practices can also be found in standard agroforestry texts (Nair, 1993; Young, 1997; Huxley, 1999).

Trees and soil fertility

Whatever the reasons farmers have for planting or protecting trees in a specific case, they nearly always fulfil several functions simultaneously. Trees may have been planted on a hillslope to produce timber or fruits, but they may also protect the soil from being eroded. Trees planted or retained for fodder are often nitrogen-fixing and may improve nitrogen availability in the soil. Similarly, trees that have been allowed to regenerate in a riparian zone because of environmental regulation, for firewood production or simply an appreciation of their scenic value may also filter nutrients from runoff water, thereby retaining them in the land-use system and protecting the river from eutrophication.

This book is about the effects of trees on soil fertility. Soil fertility is defined here as the ability of a soil to serve as a suitable substrate on which plants can grow and develop. Fertile soils facilitate root development, supply water, air and nutrients to plants, and do not have pest and disease burdens that result in catastrophic impacts on the plants that are being grown. Maintaining soil fertility is the basis of all forms of sustainable land use, that is, land use that remains productive in the long term. If fertility has fallen below a critical level through long-term agricultural use without replacement of nutrients or as a result of erosion, or if it is naturally very low, the replenishment of soil fertility may be a precondition for productive agriculture.

Tropical soils are not generally infertile, but infertile soils are very common in the tropics. Moreover, recent research has provided evidence that soil fertility is decreasing in many farmed areas in the tropics. Nutrient budgets which were established at different spatial scales in sub-Saharan Africa, Ecuador and small farms in Costa Rica showed net nutrient losses from agricultural soils because of off-take in crop yields, leaching, erosion,

runoff and gaseous losses, which were not matched by nutrient inputs from mineral and organic fertilizers, atmospheric deposition, biological nitrogen fixation and, in flooded or irrigated areas, sedimentation (de Koning *et al.*, 1997; Smaling *et al.*, 1997; Stoorvogel and Smaling, 1998). Negative nutrient budgets like these are especially threatening for the fertility of soils whose nutrient stocks are already small, such as sandy soils with low organic matter contents, which are widespread in African savannas, or acid soils, which occupy vast areas of the humid tropics of Latin America, Africa and Asia (von Uexküll and Mutert, 1995). Accordingly, long-term fertility studies on farmers' fields in African savannas have revealed evidence of widespread chemical and physical soil degradation, including negative soil organic matter and nutrient balances, although these have not always immediately been translated into declining crop yields (Pieri, 1989). Low and declining soil fertility are recognized by many tropical farmers as major constraints to agricultural production (Smaling *et al.*, 1997). It can be expected that projected growth of human populations in tropical countries will further aggravate these problems, especially when population pressure increasingly obliges farmers to cultivate fragile and naturally infertile soils which are particularly prone to degradation.

Both scientific research and farmers' observations clearly point to the need to improve current farming practices with respect to their ability to increase and sustain soil fertility and agricultural productivity. The green revolution attempted to increase agricultural productivity in the tropics through increased inputs of mineral fertilizers, pesticides and new crop varieties. Although the successes were sometimes spectacular on relatively fertile land with good infrastructure, large numbers of small farmers in marginal environments were bypassed by these developments because they could not afford the necessary investment in new seeds and chemical inputs. Gradually, it has become understood that more accessible means are needed to enable many small farmers to feed their families and raise their living standards, and it has been suggested that this is most likely to come about from a thorough understanding of the biological bases of soil fertility (Woomer and Swift, 1994). Trees with their numerous beneficial effects on soil fertility play an important role in this strategy.

The fundamental assumption in agroforestry, that the integration of trees into farming systems and landscapes can increase soil fertility, productivity and sustainability, was initially based mainly on the observation that soil under forest vegetation generally remains fertile and that tree fallows are able to regenerate degraded soils, as occurs in shifting cultivation systems (Nair, 1984). Subsequent scientific research has increasingly produced insights into the mechanisms through which trees improve soil fertility, though many key processes have still not been fully quantified. Agroforestry practices have been shown to influence chemical, physical and biological components of soil fertility. Trees can improve the

nutrient balance of a site both by reducing unproductive nutrient losses from erosion and leaching and by increasing nutrient inputs through nitrogen fixation; they can improve soil structure, water-holding capacity and crop rooting volume; and they can increase the biological activity in the soil by providing biomass and a suitable microclimate. However, a better understanding of the interactions between trees and soils has also helped to keep expectations at a realistic level and to recognize what agroforestry can and cannot achieve. Many commonly measured nutrient fluxes in agroforestry systems are part of the internal nutrient cycling within a system and do not change its overall nutrient budget. If a site is deficient in nitrogen and phosphorus, leguminous trees may be able to increase the availability of nitrogen through biological nitrogen fixation, but phosphorus may have to be added from external sources.

It has also become increasingly clear that the intensity with which trees and tree–crop associations influence soil fertility differs widely between agroforestry practices, even if the processes are similar in principle. For example, the nitrogen fixation and biomass production of relatively few leguminous trees dispersed in a crop field will be much less than those of a closed stand of these trees in a planted fallow. Also, most trees will take up some of their nutrients from the subsoil and deposit them in surface soil through leaf litter and root decay and thus act as a nutrient pump. However, for some combinations of species managed in particular ways, this process may be important, whereas for others the effects may be too small to be of much consequence. When designing or improving agroforestry techniques, it is therefore important that the technique is matched with the fertility problems that are seen as priorities at a given site, rather than assuming that every type of agroforestry will improve soil fertility in general.

Matching an agroforestry technique to the biophysical aspects of a site is necessary but not sufficient to ensure adoption; it also has to be compatible with the views, experiences, traditions and economic capacities of the farmers. Beneficial effects of trees on soil fertility are often only perceptible after several years and small farmers often cannot afford to invest in tree planting and tending without receiving an immediate return. Some techniques may require more time for pruning or biomass transport than the farmer can afford, especially when these activities are necessary at times when sowing, weeding or harvesting of crops are more urgent needs. It is also important to recognize that sustainable production from the same piece of land on the basis of stable soil fertility is not always a primary objective of the land user. Where the effort for clearing a new piece of land is smaller than that of maintaining the productivity of the already cleared plot, or where land clearing is a way of establishing ownership rights or is advantageous for other reasons, investments in sustainability may not have a high priority. Similarly, where runoff and

erosion from hillslopes benefit valuable crops in the valley below, soil degradation on the slopes may be seen as an acceptable price to pay for high productivity in the valley. It is thus clear that progress in agroforestry depends on a thorough understanding of both the biophysical and socioeconomic dimensions of farming systems at a range of scales.

1.2 Objectives and Structure of the Book

This book provides an overview of the principal concepts that have been developed over the past decades concerning the effects of trees on soil fertility and an in-depth discussion of the methodological approaches that are appropriate to their study. It has been written mainly for researchers and students interested in tropical agroforestry. Case studies, examples and references have been taken mostly from the tropical agroforestry and ecology literature. However, although temperate agroforestry differs in its socioeconomic context from that in the tropics, the general biophysical processes are similar, and so most of the information presented here is also relevant to temperate conditions. Moreover, trees and soils in agroforestry are not fundamentally different from trees and soils in forests and especially in savannas, and we expect that students and researchers in forestry, agronomy and ecology will also find much useful information in the following chapters.

Included in the book are economic, chemical, physical and biological aspects of soil fertility. Because of the integrated presentation of theoretical concepts and research methodologies, the book is particularly suitable for people who intend to do practical research on the interaction between trees and soils. It will be most useful to those who already have some basic knowledge of both agroforestry and soil science, although there are comprehensive references to the literature in these areas from which such an understanding could be gained.

The chapters begin with synopses that are followed by methods sections. The synopses provide concise statements of essential background knowledge and outlines of the principal hypotheses and research results relevant to the topic at hand, and suggest key areas for future research. These sections are intended to help researchers in the identification of suitable, worthwhile topics for their research, and for students to see individual research results within the context of the larger body of knowledge and hypotheses relating to tropical soil fertility and agroforestry. The methods sections not only include those methods that are currently used in agroforestry, but also methods whose utility has been demonstrated in other fields and that could and should be applied in agroforestry studies in the future.

This is not a methods book in the conventional sense, as it does not provide detailed field and laboratory descriptions of research methods. It has been produced to augment rather than to replace established methods texts, such as *Tropical Soil Biology and Fertility: a Handbook of Methods* (Anderson and Ingram, 1993), by furnishing a broader discussion of the scientific background of soils research in tropical agroforestry and the range of available research methods. The book also provides extensive reference to the relevant methodological literature, both from agroforestry and from soil science in general.

The book starts with a chapter on the economics of soil fertility management and agroforestry practices, in which concepts for the analysis of farmers' decisions are presented and consequences for the adoption or non-adoption of agroforestry practices are discussed. Attention is drawn to the substantial public benefits at national and global scales that derive from the ecosystem services provided by trees. At present farmers often bear the costs of these public goods, which results in a level of agroforestry adoption that may be lower than is desirable from a societal point of view (Chapter 2). Chapter 3 presents some general grounding in appropriate methods for experimentation, sampling and data analysis for soil fertility research in agroforestry. A section on fallow experimentation is included because of the particular requirements of working with rotational systems that have distinct temporal phases and their present importance in agroforestry research. There is also a section on geostatistics, a tool that has not yet been widely used in agroforestry research but may become increasingly important in the future because of its usefulness in the analysis of spatial heterogeneity. Chapter 4 discusses the dynamics of organic matter in tropical soils as influenced by agroforestry and other land-use practices. The following four chapters are dedicated to different aspects of nutrient cycling. The synopsis section of Chapter 5 discusses the principal hypotheses concerning how trees may affect nutrient cycles and introduces the concepts of competition, complementarity and facilitation. This discussion provides a framework within which the macronutrients nitrogen, phosphorus, potassium, calcium and magnesium as well as soil acidity are discussed. The next three chapters treat nutrient cycling processes of particular relevance to agroforestry: decomposition and nutrient release from biomass (Chapter 6), nutrient leaching (Chapter 7) and nutrient capture (Chapter 8). Atmospheric nutrient inputs and nutrient losses into the atmosphere through fire are the topics of Chapter 9. Physical soil fertility is treated in the following two chapters on soil structure (Chapter 10) and soil water (Chapter 11). Five chapters are dedicated to biological aspects of soil fertility: root systems (Chapter 12), biological nitrogen fixation (Chapter 13), mycorrhizas (Chapter 14), rhizosphere processes (Chapter 15) and soil fauna (Chapter 16). Soil erosion is the topic of the last chapter (Chapter 17). Throughout the text

some effort has been made to demonstrate that the chemical, physical and biological components of soil fertility strongly interact.

Topics related to soil fertility and agroforestry that have not been included in this book include salinization and waterlogging, which have been addressed in several contributions to a recent symposium (Lefroy *et al.*, 1999); soil-related pest and disease problems, which are still a neglected field in agroforestry despite recent progress (Desaeger and Rao, 1999; Duponnois *et al.*, 1999); and allelopathy, on which a large literature exists (e.g. May and Ash, 1990; Ramamoorthy and Paliwal, 1993; Conger, 1999), although there remains a paucity of information about its practical importance in agroforestry.

Chapter 2
Economic Aspects of Soil Fertility Management and Agroforestry Practices

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2.1 Introduction

Analyses of adoption of soil fertility management and agroforestry practices by farmers in tropical countries are relatively sparse. They provide a patchwork picture of cases where adoption occurred relatively rapidly and was quite widespread and other cases where adoption did not occur on any significant scale. This chapter deals with the economic concepts that can be used by scientists interested in increasing the probability of adoption of soil fertility management practices by farmers. It is stated in Chapter 1 of this book that: 'When designing or improving agroforestry techniques, it is ... important that the technique is matched with the fertility problems that are seen as priorities at a given site, rather than assuming that every type of agroforestry will improve soil fertility in general.' The goal in this chapter is to present economic concepts for matching soil fertility interventions to both farmers' constraints and objectives and those of society, in order to increase their likelihood of adoption. The chapter highlights key economic concepts and processes that biophysical scientists should find helpful in putting their own work into the broader context of the farmer's economic environment. It does not attempt to provide detailed discussions of economic approaches to the assessment of agroforestry practices for professional economists (economists are not the major intended audience for this book). Furthermore, such detailed discussions are not readily available in the literature, and therefore may constitute a gap which needs to be filled.

Declining soil fertility is acknowledged as a problem by the vast

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majority of farmers who experience it on their farms (see Chapter 1). It is, at the same time, a problem for society as a whole as it is related to issues of agricultural sustainability, soil biodiversity, carbon sequestration and watershed functions. This chapter focuses on the decision-making processes that determine whether an agroforestry intervention will be adopted by farmers and whether ensuing levels of adoption will be socially optimal.

These processes are analysed within the framework of ecological economics, which differs substantially from mainstream economics. The emerging field of ecological economics addresses the relations between ecosystems and social and economic systems. Social and economic systems are viewed as subsystems of the biosphere and thus as wholly dependent upon ecological–economic interactions. This is in direct contrast with the conventional or mainstream economic approach, which considers that all phenomena are subsumed within and obey the rules of an economic system.

In what follows, factors affecting farmers' decision-making processes regarding adoption of soil fertility and agroforestry practices are first highlighted. A brief discussion of agricultural systems hierarchy serves to set the context within which farm-scale decision-making processes are analysed. Finally, economic processes relevant at the regional and global scales are discussed.

2.2 Factors Influencing Farmers' Decisions About Soil Fertility Management Practices

The decision to adopt a given soil management technology is made by an individual farmer on the basis of a number of factors which the farmer integrates into a framework driven by the farmer's production objectives. Social scientists who have analysed farmers' decision making in the tropics have shown that farmers think in a systemic fashion. Decisions regarding a given field or a given practice, such as soil fertility management and agroforestry, are thus not made in isolation. These decisions are made within the context of the whole farm and of the totality of the resources and assets available to the farmer. These resources and assets include: (i) labour (family labour plus hired labour if sufficient cash is available); (ii) cash to buy fertilizer and other chemicals; (iii) their entire landholding and the different fields comprising it; (iv) purchased assets such as implements, machinery, animal traction; (v) access to water (either on farm or off farm); and (vi) access to other off-farm resources (such as communal resources, forested lands and woodlots).

Farmers focus on the trade-offs between the efforts they have to make to meet their production objectives (being able to produce enough food

for the family, being able to produce a surplus and to sell it) and the payoffs they expect from these efforts. They consider the totality of their holding and its various current and potential uses *vis-à-vis* their production objectives and weigh these in terms of the outcomes they expect when combining their resources into different practices (including soil fertility management).

Farmers also have to factor into such decisions different groups of markets and different types of customs. They need to consider markets for agricultural commodities (local, regional, national and international if relevant), since the sale of their surplus production, over and above what they need to produce to feed their family, depends on these markets. They also have to take into account markets for inputs. These determine the costs they will have to incur for their use of: (i) labour, be it hired or family labour; (ii) land, especially if they rent some of their fields; (iii) capital, especially if they borrow money; (iv) implements and machinery; and (v) water and other resources. The level of income of farmers is determined by these two groups of markets. Finally, the actual purchasing power of this income is dictated by markets for consumer goods (such as clothing and medicines) and by government policies regarding public infrastructure (such as means of transport and their costs, costs of education, health and electricity).

Social customs and norms influence a number of the elements that farmers need to integrate in their decisions. These customs and norms determine farmers' access to many natural resources, as well as human labour, through the prevailing land and tree tenure system, and the regulations concerning access to off-farm resources (water, communal resources such as forests and woodlots), and access to non-family labour. In some societies in western Africa for instance, rules of access to labour are extremely complex and based on what anthropologists call reciprocal relations.

It is important for scientists undertaking research on the development of soil fertility management practices to be cognizant of the fact that farmers' decisions to adopt such practices are complex and driven by the conjunction of the above factors. Scientists need to understand the basic principles at play in such decisions in order to design interventions that match farmers' constraints and are therefore adoptable.

The various types of factors influencing farmers' decisions are depicted in Fig. 2.1. Figure 2.1 also illustrates the fact that these factors are determined at different spatial scales. Although some of them are clearly determined at the farm scale (e.g. family labour and soil fertility status of the fields) others are determined at the landscape and community scale (e.g. access to off-farm resources) or at the regional and national scale (e.g. government policies concerning rural infrastructure and markets for consumer goods).

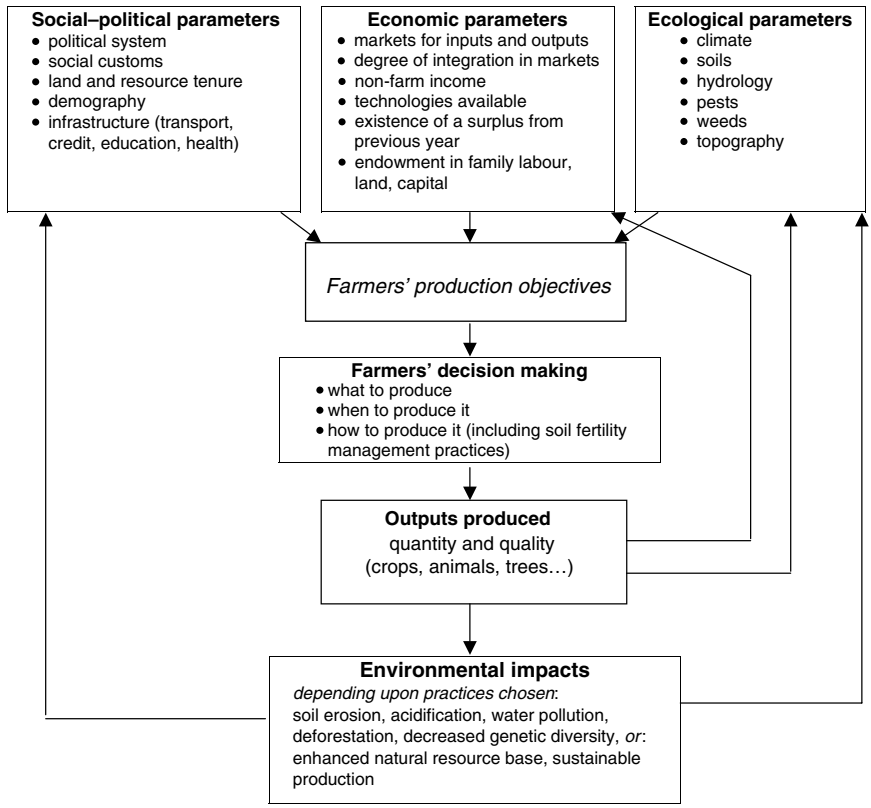


Fig. 2.1. Context of farmers' decisions to adopt.

Finally, Fig. 2.1 shows that a decision to use specific practices, as per these factors and per the farmer's production objectives, have specific outcomes for the farming household and may also have off-farm consequences. The off-farm consequences include, for instance, the forest degradation that would result from farmers deciding to ameliorate the effects of decreasing soil fertility on their fields by transferring forest soil to their land, as occurs in parts of India. Another example would be the increased water sedimentation for downstream farmers resulting from a decision to clear secondary fallow land for agriculture by upstream farmers in northern Thailand.

The purpose of this section is to debunk some often-held myths about farmers' decisions concerning soil management in tropical countries. The following points are stressed. First, farmers in tropical countries are as rational in their decision making as any other persons or stakeholders in any country. Secondly, they generally have a different perspective from

that of scientists when considering improved soil management practices. Theirs is a system perspective whereas scientists often adopt a reductionist perspective when designing technologies. This may explain why rates of adoption of these technologies by farmers are often a disappointment to scientists who have not factored into their technology design all the relevant constraints faced by the farmers. Thirdly, farmers' decisions to

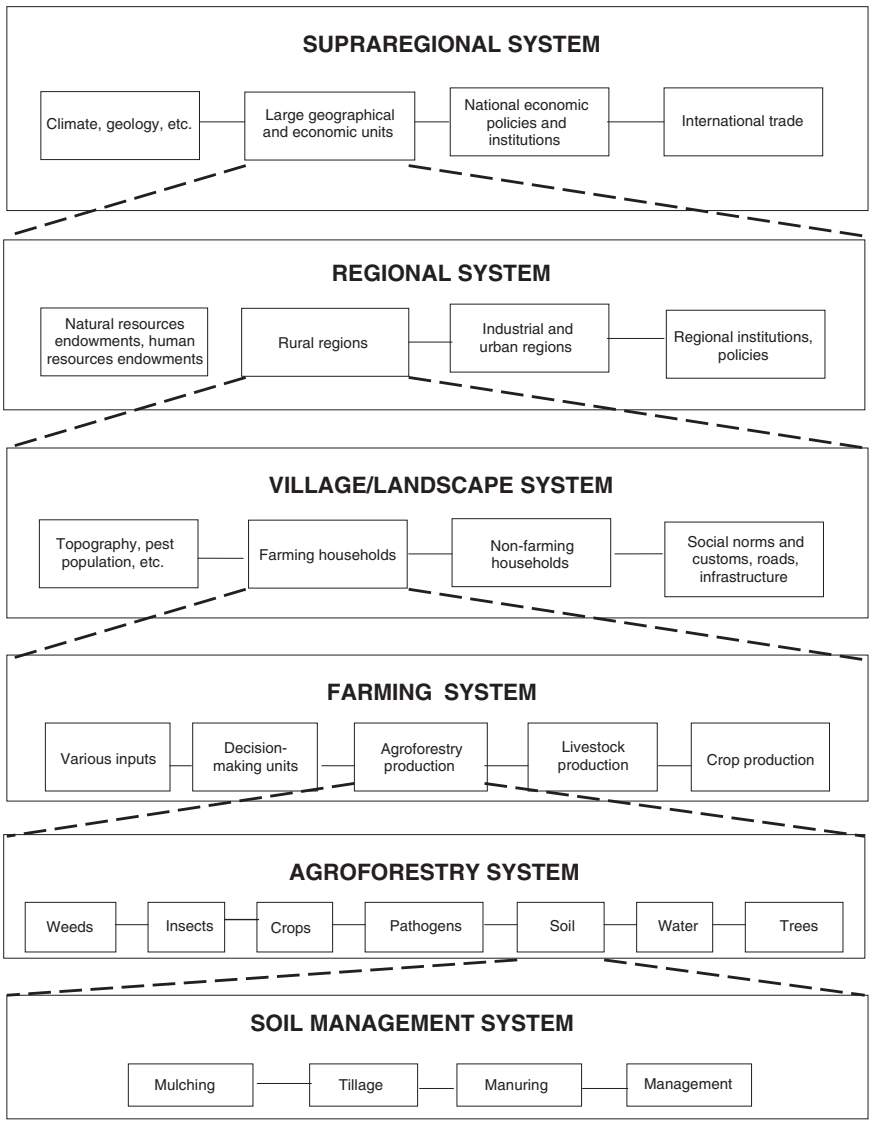


Fig. 2.2. Hierarchy of systems within which farmers make decisions.

adopt are constrained by various factors, determined at different spatial scales; likewise these decisions to adopt can have consequences at spatial scales beyond the farm scale.

2.3 Hierarchy of Agricultural Systems as a Background to the Understanding of Farmers' Constraints

Figure 2.2 represents a spatial hierarchy of agricultural systems within which farmers' decisions are made. The highest level in the hierarchy, supraregional systems, occupies the largest land area and can transcend national boundaries. Macroeconomic processes, as well as certain geological processes, are best understood at this level. The lowest level (soil systems) covers the smallest spatial unit and is the level at which specific biological processes such as nutrient uptake may be investigated. A basic rule in systems theory is that systems at level n are constrained and controlled by systems at level $n + 1$, and in turn they constrain systems at level $n - 1$ (Allen and Starr, 1982).

Farmers (operating at the farming system level) thus have to take the environment provided by the village or landscape level as a constraint in their decisions regarding soil fertility practices. Likewise, the village or landscape scale is constrained by regional system variables that operate themselves within the confines of supraregional variables. Consequently, farmers integrate a wide range of ecological, social and economic parameters belonging to levels higher than the farming system in their decisions to adopt soil fertility management and agroforestry practices.

Decisions made at the farming system scale have repercussions at the same scale, as well as at lower and higher scales in the hierarchy. These are mediated through various economic and biological processes, such as nutrient cycling and the market mechanism. Because these processes transcend farm boundaries, it is helpful to establish a distinction between the economic processes that occur at the farm scale and those that are manifest at the landscape or watershed and global scales. Even though soil fertility management and agroforestry practices are very localized interventions on farmers' fields, it is essential for scientists to realize that the key processes of relevance to their adoption occur at the farm, regional and global scales.

2.4 Anatomy of a Decision at the Farm Scale and Economic Methods for Understanding such Decisions

The decision by an individual farmer to adopt a given soil fertility management or agroforestry practice is made within the frame of the

factors shown in Fig. 2.1. More specifically, such decisions are based on farmers' production objectives and farmers' perceptions of the advantages and disadvantages of a given practice. These advantages and disadvantages are all the monetary and non-monetary costs and benefits of a practice, as perceived by farmers.

All these costs and benefits are relative to, or determined by, the existing land tenure system and markets. For instance, Unruh (2001) has shown that in post-war Mozambique, where land rights are unclear and ambiguous, land disputes are very common and costly. Agroforestry trees, in particular older cashew trees (*Anacardium occidentale*), are, however, considered as evidence of land ownership. In such an institutional context, these trees have a substantial non-monetary benefit; they serve to clearly establish stronger land claims for farmers (Unruh, 2001). Notions of costs and benefits are thus highly relative concepts in economics. What is a benefit in a given institutional context, such as trees that establish land rights in Mozambique, may not be so in countries where land rights are unambiguous.

In what follows, the basic economic principles and processes at play in farmers' decisions to adopt or not to adopt are analysed, and examples from actual economic analyses are given to illustrate the argument. The 'real-world' costs and benefits of a given agroforestry practice in a given location are highly site specific and variable across farms. This is a direct consequence of the complex range of factors that determine the value of each cost and benefit. It is thus very important for scientists interested in understanding farmers' decisions to be aware of the economic processes that guide these decisions. Without such an understanding, empirical evidence of the profitability (or lack thereof) of specific agroforestry options may appear very confusing.

Principles of private cost–benefit analysis and net present value

One of the simplest ways to assess these costs and benefits is to use private cost–benefit analysis. This analysis consists of comparing the flows of benefits and costs generated over time by the adoption of a given agroforestry practice. Only the costs and benefits that are relevant to a given farmer are taken into consideration, and they are valued at the market prices faced by the farmer. No attempt is made to adjust costs and benefits for market and government failures. Nor is any attempt made to take social costs and benefits (externalities and environmental benefits) into consideration. Pagiola (1994) and Sugden and Williams (1978) provide excellent introductions to cost–benefit analysis for non-economists. Gittinger (1982) is one of the classic texts on cost–benefit analysis, along with Mishan (1976). Both provide much detail about the approach, its basic

assumptions, and various methods of computing costs and benefits. Both texts are written for professional economists.

The basic principle in private cost–benefit analysis is simple. The net value, in today's dollars, of the future flows of benefits and costs of an agroforestry option is computed. In economic language, this is called the *net present value* of an agroforestry option. This net present value (NPV) is defined as:

$$\text{NPV} = \sum_{t=1}^{n} \frac{B_t - C_t}{\left(1+r\right)^t} \tag{2.1}$$

where: B_t = benefits in year t; C_t = costs in year t; r = discount rate, that is, the rate at which benefits and costs incurred in year t have to be discounted to arrive at their value in today's dollars; and n = number of years during which the agroforestry option will generate benefits and costs.

In what follows, the concepts of costs and benefits are further discussed, as well as the concept of discount rate.

Monetary and opportunity costs of adoption

The generic soil fertility and agroforestry practices that are discussed in the other chapters of this book include improved fallows (tree–crop rotations), tree–crop associations (such as hedgerow intercropping and shaded perennial crops), contour hedgerows and boundary plantings. Each of these options entails various monetary and non-monetary costs for farmers. Therefore:

$$C_t = mc_t + oc_t \tag{2.2}$$

where mc_t = sum of monetary costs in year t, and oc_t = sum of opportunity costs in year t.

The monetary or out-of-pocket costs (mc_t) are quite straightforward. They consist of: (i) the costs of buying seeds and/or seedlings (or the costs of having an on-farm nursery for the tree seedlings), and whatever other inputs such as fertilizer are used, and (ii) the costs of hiring additional labour (if family labour is insufficient) for planting the trees, weeding the trees, pruning them, and applying or incorporating tree prunings to the soil (see Table 2.1). The most meaningful way of assessing these costs is on the basis of on-farm experiments or under actual farming conditions, through on-farm monitoring of farmers' practices. Monitoring the use of hired labour throughout the year, and throughout the lifespan of an agroforestry option is not an easy task, and is costly when this lifespan is long. There are therefore relatively few examples of private cost–benefit

Table 2.1. Generic on-farm costs of agroforestry practices.

- Monetary costs of additional hired labour for planting tree seedlings/seeds, pruning the trees, weeding the trees (most agroforestry options)
- Monetary cost of purchase and transport of seeds and seedlings and fertilizer (if applied; most agroforestry options)
- Opportunity cost of not using a field or part of a field in another way (for improved fallows, intercropping, alley cropping and trees on contour ridges)
- Opportunity cost of family labour used (forgone participation in other farming activities; most agroforestry options)
- Opportunity cost of possible losses in germination of crops (for agroforestry options requiring the application of tree residues to crops)
- Opportunity cost of lower crop yields due to competition from trees (for intercropping, alley cropping and parklands)

analyses that use such 'real-world' data. Many analyses rely on on-station experiments and on extrapolations of on-station data to on-farm conditions. Dewees (1995) provides an interesting analysis of the costs to farmers in Malawi of intercropping maize with *Faidherbia albida*, and of alley cropping *Leucaena leucocephala* with maize.

The non-monetary or opportunity costs (oc_t in Eq. 2.2) are not actual disbursements for farmers, but are nevertheless very real costs which farmers take into consideration in their decisions. They consist of all the opportunities for generating benefits that a farmer gives up when choosing a given agroforestry option (see Table 2.1). The appropriate baseline for such comparison is the best alternative practice that the farmer could have chosen in lieu of this agroforestry option. If there is an alternative practice that would bring higher benefits to the farmer than agroforestry, then it is highly unlikely that the farmer will choose agroforestry, unless agroforestry also brings about significant intangible social benefits (such as increased social status in the community) for the farmer.

Two principal types of opportunity costs of agroforestry options are those of labour and land. When family labour is used for planting hedgerows, for example, the opportunity cost is equal to the benefit that this family labour could have generated if instead of planting seedlings it had been engaged in the best alternative, such as planting or weeding food or cash crops. The opportunity cost of family labour can sometimes be a major constraint to the adoption of agroforestry practices because:

- the extra labour required for implementing some of these practices may be needed at a time of year when all household members are occupied in tasks given a high priority (e.g. production of food crops); and/or
- available family labour is decreasing in some areas due to HIV/AIDS; and/or

- in some countries such as Kenya there is a fairly strict division of on-farm responsibilities between females and males, so that even if female labour is plentiful some tasks such as pruning trees in hedgerows may not be done because male labour is insufficient (see Swinkels and Franzel, 1997, for details).

In such cases, private cost–benefit analysis in itself may not be sufficient in capturing these non-quantifiable constraints, and farmer surveys will be needed to complement the picture painted by private cost–benefit analysis. It could indeed be the case that a given agroforestry option, although economically profitable, is at the same time not really feasible, and therefore will have a low adoption rate, for the reasons just mentioned.

Whereas monetary costs of adoption are simply assessed by their market value (e.g. cost of one seedling times number of seedlings purchased; hourly wages paid to hired labour times number of hours worked), opportunity costs are more difficult to assess. In the case of family labour, economic theory indicates that the prevailing wage rate times the number of hours worked by family labour should be used to assess this cost. This is because it is assumed that, if family labour had not been working on the farm, it could have been engaged working as hired labour on other farms. When rural unemployment is high, however, it is doubtful that it would have been feasible for family members to be thus employed in the agricultural sector. As a rule of thumb, the higher the rate of unemployment in an area, and the more difficult for family members to travel within this area (because of geographical isolation or bad transport networks), the lower is the wage rate that should be used to assess the opportunity cost of family labour (see Pagiola, 1994, for a clear discussion of these issues).

The opportunity cost of an agroforestry option in terms of the land area it occupies (entire field or portion of fields) is easier to assess. It is equal to the benefits the farmer would have gained from the best alternative land use. This is generally the dominant or traditional land use in an area, such as the cultivation of maize with a very low level of fertilizer in the case of improved fallows in western Kenya. In such an example, the opportunity cost of the land under improved fallows is equal to the net benefits the farming household would have received from the traditional maize cultivation system on a per hectare basis, times the land area under fallow. In some instances, the best alternative to the agroforestry option under analysis may be a different soil fertility enhancement method that farmers could choose to use (e.g. green manuring with legumes). In this event, net benefits from manuring with legumes on a per hectare basis times the land area under fallow will be equal to the opportunity cost of a fallow practice.

Not only are these various costs (represented in Table 2.1 and equal to C_t in Eq. 2.2) entirely borne by the farming household, but also the household must start paying for them from the moment a practice is chosen for implementation. In addition, some of these costs will recur over a number of years. In some of the few assessments of actual costs of adoption incurred by farmers published in the literature, total costs of adoption decrease very substantially between year one and the following years, and are, during the first years, significantly higher than the costs of implementing farmers' current practices. This was the case, for instance, for hedgerow intercropping (maize with *Leucaena leucocephala* or *Calliandra calothyrsus*) in the highlands of western Kenya. The total costs of adoption to farmers (C_t) amounted, on average, to US$422 ha^{-1} during the first year and decreased, on average, to US$276 ha^{-1} during the second year (Swinkels and Franzel, 1997). This compared with total costs of US$296 for the traditional maize system during the first year and costs of US$271 during the second year. In another example, of improved fallows in Costa Rica (with *Vochysia ferruginea* and *Hyeronima alchorneoides*), actual costs (for clearing, planting, replanting, weeding, pruning, and seedlings and their transport) were around US$1330 ha^{-1} for the first year. They decreased to US$162 ha^{-1} during the second year and US$97 ha^{-1} during the fifth year of adoption (Montagnini and Mendelsohn, 1997).

Monetary and non-monetary (environmental and social) benefits of adoption

The benefits of agroforestry practices, as they are perceived by the farmer, are generally more difficult to assess than their costs. And, furthermore, some of these benefits often start occurring after a while, so that there is a time lag between the moment when a household incurs costs in order to adopt an agroforestry practice and the moment when it starts receiving benefits from this practice.

In parallel with costs, the benefits of adoption of a practice can be monetary and non-monetary (see Table 2.2). Therefore:

$$B_t = mb_t + ib_t \tag{2.3}$$

where mb_t = monetary benefits in year t; and ib_t = non-monetary benefits in year t.

There are two kinds of monetary benefits (mb_t). The first is the value of the increased crop yields associated with a given agroforestry practice. In a maize-based improved fallow system for instance, this will be the market value of the increased maize yields following the improved fallow period. Such direct monetary benefits will occur over a period of time, that

Table 2.2. Generic on-farm benefits of agroforestry practices.

	Time[a]		
Benefits	Year 1	Years 2–5	Years 6–10
Monetary			
Increased crop yields	+	++	++
Value of various tree products	0	+	++
Non-monetary			
Increased resilience and sustainability of system through:			
Decreased risks of yield fluctuations with usual climatic variability	0	+	++
Enhanced soil resource base	0	0	+
Enhanced capacity of system to adjust to exogenous changes without generating increased flow of pollutants	0	0	+
Increased biodiversity of soil biota	0	+	++
Increased biodiversity of fauna and flora	0	0	+

[a] The time scales shown are indicative.
0, no measurable benefit; +, ++, benefit is measurable and its intensity varies from low (+) to high (++).

is, until all residual effects of the improved fallow have been exhausted. To continue with our example, it is thus necessary to assess maize yields over time in the improved fallow system (as well as maize yields over time in the traditional maize system, since their market value will constitute the opportunity cost of the land under fallow). Nominal farm-gate prices of maize over the relevant period of time also need to be obtained or predicted. Even if the maize is grown for home consumption rather than for sale, the value of this monetary benefit of adoption will be evaluated as: tonnes of maize produced multiplied by the market (farm-gate) value of a tonne of maize, divided by the relevant number of years.

The second type of monetary benefit is the market value of the diverse products that may be generated by an agroforestry practice. Examples of these products include indigenous fruits, timber, nuts, leaves that are used in food preparation, leaves, bark and seeds that have medicinal properties, poles, fodder, fuelwood. Proceeds from the sale of these products will contribute to household income and are thus a monetary benefit. If these products are not sold but are consumed by the household, their market value equivalent still represents a monetary benefit, since the household does not need to spend some of its income on the purchase of these products. In a very real way, these products directly contribute to the welfare of the household. Leakey and Tomich (1999) provide various examples of the market values of tree products from agroforestry systems,

although these systems are not restricted to those that enhance soil fertility. For all such monetary benefits, the effects of an agroforestry option on the yields of various products, from crops to medicinal products, need to be quantified on a yearly basis over the relevant time period. The value of these yields is then obtained by assessing or predicting the farm-gate prices of these products.

To use again the example of hedgerow intercropping in western Kenya, total monetary benefits increased from US$709 ha^{-1} during the first year to US$766 ha^{-1} during the second year (Swinkels and Franzel, 1997). This compared with total monetary benefits of US$707 ha^{-1} for traditional maize; these benefits remained at this level over time.

The non-monetary benefits of agroforestry practices at the farm scale (ib_t in Eq. 2.3) are particularly difficult to assess. They consist of all the improvements in soil and other ecological processes that are brought about by the agroforestry practices and are not captured by increased crop yields. In other words, to understand and assess these non-monetary benefits, one needs to first consider all the functions which enhanced soil fertility (such as increased nutrient stocks, enhanced efficiency of nutrient cycling) will have on a farm and which are over and above those already translated into increased crop yields.

One such function that farmers, including resource-poor households, particularly value is the increased resilience and sustainability, and therefore the increased risk-buffering capacity, of their systems. Recent poverty surveys undertaken by the World Bank (Kanbur and Squire, 2000) show that the poorer a farming household, the more important it is to this household to have effective strategies for coping with risk (environmental, climatic, economic). Ability to manage risks and adapt to change is actually something which poor farmers rank as a priority concern, on a par with increasing their income (World Bank, 1994; Kanbur and Squire, 2000). The next section shows that one method of evaluating this increased risk buffering capacity of agroforestry options is to use a lower rate of discount (r in Eq. 2.1).

There are many other examples of non-monetized benefits of agroforestry practices, such as increased soil biodiversity, decreased erosion, increased water infiltration and recharge of underground water table, improved soil structure, enhanced capacity of systems to adjust to change without generating increased flows of pollutants, enhanced carbon sequestration. Although some of these will eventually result in increased yields (e.g. improved soil structure), others will not have any measurable effects on crop yields (e.g. improved recharge of the underground water table).

Biological scientists have not reached a consensus about how to assess these various functions of enhanced soil fertility. It would thus be unrealistic to assume that farmers will be aware of all these functions.

Furthermore, even if they were aware of them, it is unlikely that they would factor all of them into their decisions to adopt. This is because some of these functions, such as increased soil biodiversity, are likely to be of no special interest to them.

Finally, non-monetary on-farm benefits of adoption can include aesthetic value, habitat for welcome wildlife and shade. Farmer surveys have shown that such benefits can be valued very highly by farmers (Scherr, 1995).

Table 2.2 presents generic categories of benefits of agroforestry systems for soil fertility improvements (B_t in Eq. 2.3) and illustrates a significant difference between the costs and benefits of adoption of such practices. This difference has been observed in a number of assessments of the costs and benefits of agroforestry practices (Current *et al.*, 1995; Dewees, 1995; Montagnini and Mendelsohn, 1997; Swinkels and Franzel, 1997). Most benefits are cumulative over time and reach greater amplitude 3 or 5 years following adoption whereas costs have to be borne up front, as explained previously.

Farmers' time horizons, discounting and balancing of the costs and benefits of adoption

It was seen above that there are some fundamental differences between the costs and the benefits of agroforestry systems for soil fertility management at the farm scale. The costs are entirely borne by farmers and occur from the inception of adoption of a practice. The benefits are generally cumulative over time and low during the first years following adoption. In addition, farmers are unlikely to value some of the non-monetized benefits such as increased soil biodiversity. This raises the issue of the time frame of relevance to farmers.

Recall that the NPV of an agroforestry option is represented in Eq. 2.1. This equation reflects the fact that benefits (and costs) occurring at some future time do not have the same value today as they will have in the future. Indeed, if we are asked whether we prefer to receive US$100 today or in 3 years' time, most of us will answer that we prefer to receive this sum today. This is because we can either spend the money now and immediately receive some enjoyment from it, or invest it to receive a greater amount in the future (US$100 plus interest over 3 years). The economic concept of a rate of discount (r in Eq. 2.1) is the measure which enables economists to translate future benefits and costs into today's monetary worth. It is the rate at which we need to be compensated in the future for willingly accepting not enjoying a benefit today. It is also called the rate of time preferences (see Mishan, 1976, for a detailed discussion).

As can be appreciated by simply looking at Eq. 2.1, the choice of a specific value for r is extremely important. Indeed, the value of NPV is highly sensitive to the value of r. When costs are incurred in the present and medium term, whereas benefits occur over the medium and long term, the lower the rate of discount used in the analysis, the higher the resulting NPV, and vice versa.

Eliciting farmers' true rate of time preferences is a difficult task. Economic, psychological and social factors influence this rate. To arrive at a realistic rate through very carefully designed and in-depth interviews is a research project in and of itself. Consequently, in practice analysts use a rate, chosen on somewhat arbitrary grounds, that they assume reflects as well as possible the perspective of farmers in a given community.

Resource-poor small-scale farmers in the tropics are highly vulnerable to risks (Kanbur and Squire, 2000) and generally concerned about their survival in farming from one year to the next. It is therefore likely that, even if they were aware of the medium- and long-term benefits of adoption, most farmers would value these future benefits relatively less than immediate benefits. This is because they occur over a period of time of little relevance to their immediate needs. In other words, the discount rate of relevance to farmers in tropical countries to be used in Eq. 2.1 is likely to be quite high (rates of around 20% are used in most studies). One empirical study of farmers' discount rates suggests that Costa Rican farmers discount the future at a rate of 20–25% (Cuesta *et al.*, 1994). Another study of smallholders in the Philippines indicates that 40% would be an appropriate rate of discount for these farmers (Nelson *et al.*, 1998). It should be noted that, when an agroforestry option is seen by farmers as a method for managing risk (such as farmers in Rajasthan who cope with repeated droughts by the complementary use of woody perennials and annuals), then their implicit rate of discount decreases substantially (Arnold, 1997), and rates of 5–10% can be used.

Given the difficulties associated with the choice of an exact rate of discount and the sensitivity of the resulting NPVs to this choice, a good analytical practice is to use a range of rates. The corresponding range of NPVs will highlight the threshold rate of discount at which an agroforestry option becomes privately profitable for farmers.

The relatively few empirical assessments of the NPV of different agroforestry practices generally indicate a positive NPV. For instance, in the 21 agroforestry projects implemented in eight countries of Central America and the Caribbean that were reviewed by Current *et al.* (1995), NPVs of trees with crops, alley cropping, contour planting, perennial crops with trees, homegardens and taungya systems were all positive, whereas the NPV of woodlots was negative at a 20% discount rate, indicating net economic losses for farmers. In another study on the loess plateau in China, the NPV of an agroforestry intervention consisting of apple

orchards on bench terraces was positive, and was also about 100% higher than the NPV of soil conservation practices without a tree component (Lu and Stocking, 2000).

Comparing the NPV of an agroforestry option to that of alternative options for farmers is an important step in understanding decision making at the farm scale. Various studies suggest that agroforestry systems typically have higher NPV than current or traditional practices (e.g. Current *et al.*, 1995, for Latin America and the Caribbean and the above example from China), with some notable exceptions in Africa (e.g. Dewees, 1995, for intercropping and alley cropping in Malawi; Drechsel *et al.*, 1996, for improved fallows in Rwanda; Swinkels and Franzel, 1997, for alley cropping in Kenya).

In addition to ensuring that the over time private profitability of an agroforestry option is compared to other options of relevance to farmers interests, it is also important to assess the sensitivity of this measure of profitability to changes in some variables. Sensitivity analysis consists of varying different parameters in Eq. 2.1, such as the price of labour, the yields and prices of the crops produced, to assess the effects of these changes on NPV (see Gittinger, 1982, for further details). The data used to assess these parameters have a built-in uncertainty, given the paucity of relevant secondary databases in tropical countries, the unreliability of existing data and the fact that primary data are costly and often difficult to collect (see Lele, 1991, for a discussion of these issues). It is therefore important to investigate the effects of changing the values of these parameters, within reasonable bounds, on the results of the analysis to better appreciate the robustness of the profitability of an agroforestry option. The study by Current *et al.* (1995) indicates that the positive and high NPVs obtained for the vast majority of agroforestry practices in various Latin American and Caribbean countries were most sensitive to changes in yields and product prices. In the African context, it would appear that results of NPV are most sensitive to changes in adoption costs and yields (e.g. Dewees, 1995; Drechsel *et al.*, 1996).

The absolute value of the NPV for an agroforestry option thus provides much information about the private profitability of the option, over its life span. The quality of the information is increased when a range of discount rates is used, when NPVs between different options are compared and when sensitivity analysis is undertaken. The concept must be further interpreted and analysed in order to also provide information about the adoptability of an agroforestry option, in addition to its private profitability.

Interpretation of results from private cost–benefit analyses

Since NPV is an aggregate estimate of profitability over time, it is essential to identify the length of time during which net losses will be incurred by adopters before NPV starts becoming positive. This is called the *break-even point* of an agroforestry option. The evidence reported in the literature indicates that many agroforestry practices have break-even points longer than 1 year. An example concerns the adoption of trees on contour lines in Java. It takes a minimum of 5 years for the cumulative on-farm benefits to exceed the initial cost outlays in these agroecosystems (Barbier, 1990). In the example of adoption of improved fallows in western Kenya, it takes 4 years for this to happen (Swinkels and Franzel, 1997). In their review of multiple agroforestry practices in several countries, Current *et al.* (1995) found break-even points ranging from just under 2 years (for alley cropping) to more than 9 years (for woodlots). Adoption of agroforestry

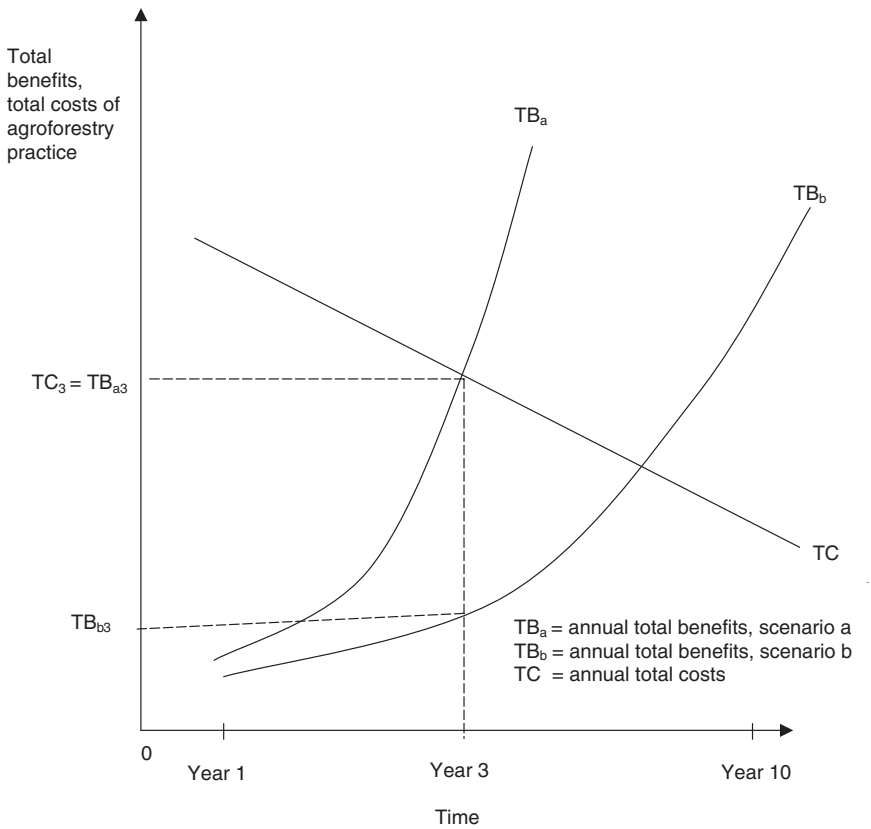


Fig. 2.3. Farm-scale costs and benefits of agroforestry adoption over different time periods.

options that entail losses during 2 to 5 years (these are the most common break-even points reported in the literature) thus requires that farmers have the financial ability to absorb these losses. Clearly, not all resource-poor farmers in tropical countries will have such an ability.

The soil fertility management and agroforestry practices that have the highest likelihood of being adopted by these farmers are those that result in sufficiently significant short-term benefits to compensate for the costs of implementation. This is summarized in Fig. 2.3, in which these costs of implementation are shown as a decreasing function over time. The corresponding benefits curve however, increases exponentially with time, for the reasons mentioned above.

Two scenarios are represented in Fig. 2.3. In scenario a, total benefits (TB_a) increase rapidly over time whereas in scenario b they increase relatively slowly. If the planning horizon of the farmer is up to 3 years and if scenario a prevails, then adoption of agroforestry practices can be expected. If scenario b prevails and the farmers' planning horizon is around 3 years, then adoption is unfeasible because, over this time horizon, total benefits (TB_{b3}) remain below total costs. This balancing of monetary and non-monetary, present and future, on-farm costs and benefits over the duration of the farmer's planning horizon is a fundamental economic process affecting soil fertility management at the farming system scale. The few economic analyses of agroforestry practices for soil fertility management that rely on actual on-farm data provide an illustration of this when they stress that a constraint to adoption is that farmers often do not have sufficient capital to be able to withstand the few years of low, or even negative, profits which precede the years of relatively high profitability (e.g. Scherr, 1995; Grist *et al.*, 1999).

It is enlightening to note that even in the economic context of the USA, where farmers receive government subsidies (called cost-sharing), tax credits and deductions for adopting agroforestry practices, rates of adoption of agroforestry practices have been lower than expected for essentially the same reasons. A survey of farmers in the southern USA revealed that, although these farmers were aware of the environmental benefits of agroforestry, 'considerable uncertainty remained' about the potential profitability of various agroforestry systems in the minds of these farmers (Zinkhan and Mercer, 1997).

In summary, the fundamental economic processes that occur at the farm scale are as follows.

- Farmers bear all the costs of adoption of improved agroforestry and soil fertility management practices, and these costs start occurring from inception of adoption and continue over time.
- Farmers accrue the monetary benefits of adoption and some of its non-monetary benefits.

- These benefits are cumulative over time and tend to be relatively low during the initial years following adoption.
- The time lag between costs and benefits of adoption for farmers, coupled with the fact that small-scale resource-poor farmers have short-term planning horizons and high discount rates, implies that rates of adoption can be very disappointing to scientists and policy makers.
- A major implication for scientists is that it is essential for them to design practices which minimize up-front costs of adoption (labour, capital and land).

The simplest and most effective economic method available for quantifying these processes for specific agroforestry practices is private cost–benefit analysis. It encompasses the computation of the NPV of a practice, from the perspective of an individual farmer. Very useful insights into the probability of adoption of a practice can be gained by: (i) comparing the NPV of an agroforestry option with the NPV of other land-use options of relevance to farmers; (ii) undertaking a sensitivity analysis of NPV; and (iii) estimating the break-even point of an agroforestry option.

Other economic methods: modelling

Once the above processes are quantified, there are a few additional methods that can be used for simulating the profitability, adoptability and biophysical consequences of agroforestry practices. These methods all entail some form of modelling of the economic processes just discussed, in relation either to biophysical processes or to various social and cultural variables. Most of them have been developed very recently and have been validated in only one set of circumstances. They are mentioned here only for reference purposes as they lie outside the scope of this book.

An example of a bioeconomic model, that is, a model that integrates economic and biophysical variables and processes, is provided by Grist *et al.* (1999), who used SCUAF, an agroforestry biophysical model, in combination with economic spreadsheets. Another example can be found in Shepherd and Soule (1998), who have built a farm simulation model which simulates the effects of different land-use management strategies, including agroforestry options, on soil processes and nutrient-limited plant production and on profitability.

Adesina *et al.* (2000) provide an example of a model (Logit) that explains actual farmers' decisions to adopt alley farming on the basis of a number of explanatory variables. These variables reflect the land tenure system, the socioeconomic characteristics of farmers (e.g. level of education, membership in farmers' association) and village-specific

characteristics (e.g. land pressure, erosion index and importance of livestock in the village).

2.5 Landscape and Global Scales: Soil Fertility and Agroforestry Trees as Part of Natural Capital

It was shown in Section 2.3 that farmers' decisions at the farm scale not only are shaped by variables determined at higher levels than the farm in the hierarchy of systems but also have consequences at these higher levels. Two concepts from ecological economics are particularly useful in understanding these interactions between scales. These are the concepts of natural capital and of ecosystem services.

It has been demonstrated that soil nutrients and trees are part of natural capital (Izac, 1997, for soil nutrients; Izac and Sanchez, 2001, for agroforestry trees). Natural capital comprises all the natural resources that provide useful goods and services for mankind. More specifically, natural capital is defined as stocks of resources generated by natural biogeochemical processes and solar energy that yield useful flows of services and amenities for the present and into the future (Izac, 1997). Natural capital generates ecosystem services which are the processes ensuring the productivity, integrity, maintenance and resilience of ecosystems.

The ecosystem services generated by soil nutrients include nutrient cycles, soil fertility, plant nutrition and carbon sequestration. The ecosystem services generated by agroforestry trees include, for instance, erosion control, water cycling, pest and disease control, and biodiversity.

An important characteristic of ecosystem services is that they occur over different spatial and temporal scales (Izac, 1997). Carbon cycling, for instance, is a global cycle related to climate change, taking place over the atmosphere, soils and ocean-bottom sediments, and doing so over relatively long periods of time. At the plot scale, carbon is linked to soil fertility. Table 2.3 illustrates the principal ecosystem services of trees at different spatial scales.

Since soil nutrients and trees (natural capital) generate different ecosystem services at different spatial scales, different members of society will be affected by these ecosystem services. Likewise, the management of these ecosystem services and changes in this management affect these different groups in society. When the non-monetary benefits of soil fertility management through agroforestry were discussed, it was pointed out that farmers may be quite indifferent to some of these benefits or ecosystem services (e.g. soil biodiversity and carbon sequestration). These benefits are, however, valued by other members of society. National societies in tropical countries do value sustainability of food production and

Table 2.3. Principal ecosystem services of agroforestry trees at different scales.

Scale	Ecosystem functions
Farm	Food production Nutrient cycling Erosion control Water cycling Genetic diversity Microclimate regulation
Watershed/village/landscape	Decreased poverty Erosion and sedimentation control Water cycling Refugia, pollination, biological control (landscape patches)
Region	Decreased poverty Decreased deforestation and desertification Biodiversity Water cycle
Global/supraregional	Greenhouse gas regulation Climate regulation Biodiversity Rural poverty alleviation

biodiversity, for instance, since most countries have food security and biodiversity strategies and the global society does value carbon sequestration, as demonstrated by the fact that there is an international market for sequestered carbon.

From an economic perspective, such benefits of the ecosystem services of agroforestry systems for soil fertility management are positive environmental externalities. These are defined as follows. Investments by farmers in agroforestry systems trigger flows of non-monetized benefits accruing to different groups in society, called environmental externalities. Farmers accrue some of these benefits, largely those related to increased risk buffering capacity and sustainable food production (in addition to accruing the monetary benefits of agroforestry). National society will receive the benefits of decreased rural poverty, watershed protection, increased biodiversity, more sustainable agricultural production and increased food security. Global society will enjoy increased carbon sequestration and biodiversity benefits.

These various environmental externalities are likely to be highly valued by national and global societies because these have a longer planning horizon than individual farmers. The relative significance of

ecosystem functions *vis-à-vis* food and income production is difficult to appraise. An attempt at evaluating these functions has, however, been made. Costanza *et al.* (1997) estimated that the global value of 17 ecosystem services for 16 key biomes is about US$33 trillion per year. This is excluding the worth of the stocks of natural capital in these biomes, as stocks of natural capital are too difficult to evaluate from an economic perspective. By comparison, the world gross national product (GNP) is only worth some US$18 trillion per year and the food produced by all croplands amounts to about US$0.13 trillion per year (Costanza *et al.*, 1997). These estimates give an indication of the order of magnitude of the value to society of the ecosystem services generated by natural capital compared to the value of agricultural production from croplands and the value of total economic production, or GNP.

The economic method most appropriate for assessing the various environmental externalities associated with agroforestry practices is social and environmental cost–benefit analysis. This consists of a complex set of economic methods (sometimes called shadow pricing) for assessing all non-monetary benefits and costs for different groups in society, in addition to the assessment of the private costs and benefits described in the previous section (see Mishan, 1976, for details). Furthermore, it requires that all price distortions due to government or policy failures be redressed as the analysis is conducted from the perspective of society as a whole (Mishan, 1976, provides the best explanation of these various methods, as well as of their shortcomings). This is expressed as:

$$\text{NPV} = \sum_{t=1}^{n} \frac{\left(A_t + I_t\right) - \left(D_t + S_t\right)}{\left(1 + r_s\right)^t} \tag{2.4}$$

where: r_s = social rate of discount; A_t = vector of all monetary benefits of an agroforestry option during year t, after adjusting prices for relevant market failures; I_t = vector of all non-monetized benefits received by different groups in society during year t, after adjusting prices for relevant market failures; D_t = vector of all monetary costs of an option, after price adjustments for relevant market failures, in year t; S_t = vector of all non-monetary costs of an option, after price adjustments for relevant market failures, in year t.

The social rate of discount (r_s) is the rate at which society is willing to trade present benefits for future benefits. Since society has a longer time horizon than individuals, this rate is generally significantly lower (e.g. 5%) than the individual rate of discount of relevance to farmers in tropical countries.

To date, social cost–benefit analysis has not been applied to the assessment of agroforestry options, probably because there are some currently unresolved methodological challenges. This is, therefore, an area

for future research. The International Centre for Research in Agroforestry, for instance, initiated in 2001 a global research project the objective of which is to assess, from an economic perspective, the various environmental externalities of key agroforestry practices.

The ecosystem services and environmental externalities associated with agroforestry systems and soil nutrients indicate that what is an optimal level of adoption of agroforestry practices from the viewpoint of farmers is a suboptimal level of adoption from the perspective of national and global society. This situation is represented in Fig. 2.4. The marginal (monetary and opportunity) costs of adoption of agroforestry practices are compared with the marginal (monetary and non-monetary) benefits of adoption received by individual farmers, those received by the national society and, finally, those received by the global society. Marginal costs of adoption are incurred uniquely by farmers, in the absence of any policy geared at implementing a cost-sharing scheme. Marginal benefits of agroforestry are higher for the national society than for individual farmers, and yet higher for the global society, for the reasons just mentioned.

Figure 2.4 shows that the level of adoption of agroforestry practices that is optimal at the farm scale (from the viewpoint of farmers) is Q_P, whereas the level of adoption national society regards as necessary is Q_N, and Q_G is the globally optimal adoption level. In the absence of any policy, farmers will adopt level Q_P, which is suboptimal from society's viewpoint

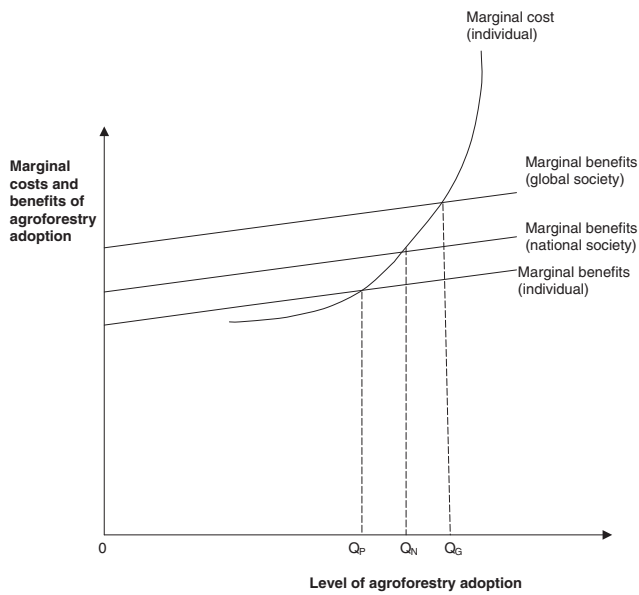


Fig. 2.4. Regional and global level trade-offs between the costs and benefits of agroforestry systems at a given point in time.

because it does not take into account most of the ecosystem services of agroforestry systems. These ecosystem services are therefore unlikely to be fully realized. These trade-offs between the individual and societal costs and benefits of agroforestry adoption (represented in Fig. 2.4) are fundamental economic processes at the regional and global scales of analysis.

The economic processes discussed in this chapter indicate that private and social interests concerning soil fertility management and agroforestry in tropical countries do not coincide. The extent of agroforestry practices voluntarily adopted by farmers (that which is in their own best interest) will almost certainly be inferior to that which is socially optimal. It will not be optimal or effective or equitable to expect resource-poor small-scale farmers in tropical countries to bear the full costs of adoption while national and global societies receive significant benefits from this adoption.

In such circumstances, providing farmers with information and advice on soil fertility management and agroforestry practices will definitely be insufficient for bringing about socially optimal adoption levels. Some policy measures will be needed for social optimality to occur. Discussions of possible policy instruments, from cost-sharing schemes, to community-based management and carbon offset mechanisms can be found in the literature and lie outside the scope of this chapter (see, for instance, Babu *et al.*, 1995; Dewees, 1995; Adesina and Coulibaly, 1998; Donovan and Casey, 1998).

The point we wish to emphasize here is that scientists working on the design of soil fertility management practices have a moral responsibility to ensure that their results are communicated to policy makers in the clearest and most effective manner possible. Sufficient information is now available about relevant economic processes at play when decisions to adopt are made by farmers, that the scientific community can no longer continue to argue that all that is needed for optimal levels of adoption to take place is good extension work and rational farmers. We now know that farmers are indeed rational and that the best extension system in the world is not set up to develop the policy instruments needed to bridge the gap between individual and societal benefits and between individual costs and societal benefits.

In summary, the key economic processes at the landscape and global scales are as follows.

- Agroforestry trees and soil nutrients are part of natural capital. As such, they generate ecosystem services at different spatial scales.
- These ecosystem services are positive environmental externalities. As such, they are non-monetized benefits of adoption that occur off farm and accrue to different groups in society (national and global society).

- These environmental externalities have a high value to national and global societies.
- Farmers do not take many of these environmental externalities into consideration in their decisions to adopt because these are off-farm benefits.
- Consequently, optimal levels of adoption from the farmer's perspective are lower than the socially optimal levels.
- Social and environmental cost–benefit analysis is an appropriate method for assessing these different processes. This is a new area of research in agroforestry.
- It is essential for scientists to communicate the results of their work to policy makers to increase levels of adoption towards socially optimal levels.

Chapter 3
Designing Experiments and Analysing Data

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3.1 Synopsis

Appropriate experimental design and analysis of data are fundamental to furthering our understanding of the impact of trees on soil fertility. Although there are many standard statistical texts that describe procedures for agronomic or forestry trials, the inclusion of one or more tree components in an agricultural context tends to increase both the spatial variability and the time horizon that have to be considered. This chapter makes reference to places where standard designs and analyses are described, but focuses on aspects of particular importance in an agroforestry context.

Given the long-term nature of many agroforestry practices, setting appropriate objectives for individual experiments is of vital importance but is seldom discussed in standard texts and is often the root cause of failure to achieve useful outcomes from agroforestry trials. Here, advice on setting objectives is given before consideration of appropriate treatment structures, layout and replication. Experiments with tree fallows are given special attention. They have become particularly important in soil fertility research over the last decade and require careful consideration because they generally involve a large number of factors, are subject to interference among plots and include treatments with different fallow lengths and hence timing and duration. The spatial and temporal heterogeneity in soils induced by the presence of a relatively few, large plants that grow over several years complicate sampling strategies, so advice is given on the most efficient sampling schemes to use to meet different types of experimental objectives.

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Collection of data is of little value unless they are appropriately analysed, but objectives for analysis are often modified as research proceeds, so a process of exploratory analysis followed by application of formal statistical tests is recommended. Particular advice is given on simple methods of dealing with repeated measurements in time and space and the appropriate statistical software to use in data analysis.

The final part of the chapter deals in some detail with methods for analysis of spatial structure. This is likely to be of increasing importance in agroforestry research, where spatial heterogeneity at various scales is increasingly embraced as an important component of agroforestry practices and landscapes, rather than being seen as something that has to be minimized. The techniques for spatial analyses are relatively new and have not yet been widely applied in agroforestry contexts. They are discussed here in sufficient detail for readers to decide how to approach analysis of spatial structure and make reference to the most appropriate literature where further details and examples are available.

3.2 Experimental Objectives, Treatments and Layout
(*R. Coe*)

The design and analysis of experiments for investigating fertility effects of agroforestry follow the principles and practices that have been developed for field experimentation over the last century or more. These principles are summarized here, with references given to more complete descriptions. Checklists of points to remember when designing an experiment may be useful and are given elsewhere by Coe (1997a, 1999). This chapter focuses on some aspects of experiments for investigating impacts of trees on soil fertility in which it can be difficult to apply these well-established principles.

Identifying the objectives of an experiment

The objectives of an experiment determine every aspect of its design, so are a key to a sound design. The objectives must be:

- *Clear*: objectives stated vaguely or ambiguously lead to confusion later;
- *Complete*: incompletely determined objectives mean that some design decisions cannot be made;
- *Relevant*: applied research has aims of solving specific problems and each experiment must have a clear place in the problem-solving strategy;
- *Capable of being met by an experiment*: not every research objective can be met by an experiment.

Much of the rest of this book is aimed at helping researchers determine what areas of research might be fruitful and how to go about making appropriate measurements of key variables. However, this is not the same as identifying objectives for individual trials that meet the criteria above and which will allow all decisions about the design to be made. Few of the texts on experimental design have much to offer on the problem of statement of objectives, even though the problems scientists encounter in many field trials originate from objectives that are confused or contradictory. The classic book by Cochran and Cox (1957) devotes half a page to the topic and more recent books do no better, maybe because there is little structured advice that can be given. A recent exception is Robinson (2000). There are, however, some common problems which can be avoided.

First, objectives as vague as 'to see what happens' cannot be used to design a trial. If the research is still at the stage of having no clear hypotheses or quantities to measure then an experiment is not (yet) needed. Secondly, careful reading of objectives often reveals them to say nothing more than 'the objective of the experiment is to compare treatments'. Objectives should be expressed as knowledge gaps to fill, that is, hypotheses to test or quantities to estimate, which lead to a definition of treatments, rather than starting with the treatments themselves. Most experiments have multiple objectives but these have to be mutually consistent. A common conflict is between objectives with a technology perspective, aimed at evaluating options for farmers, and those aimed at understanding processes. These two perspectives can often lead to different requirements of treatments and management. For example, Rao *et al.* (1998) describe a trial in which *Senna spectabilis* was deliberately used, rather than a species of greater importance to farmers in the region, because the roots were easily distinguished from those of the accompanying crops. In technology development trials, a further common difficulty is simply that of choosing where to start among the many aspects that could be studied. Cooper (1997) describes an approach to thinking through this, based on identification of critical constraints, and Franzel *et al.* (2001) discuss the appropriateness of different types of on-farm trials, with varying degrees of farmer and researcher control, for meeting different objectives.

Defining experimental treatments

The key ideas needed to define treatments for an experiment are as follows.

- *Contrasts*: most objectives can be reduced to the need to compare two or more treatments, either to detect whether a difference exists or to measure the size of the difference. Clearly the treatments to be

compared or contrasted determine the treatments required in the experiment.

- *Controls*: control treatments are nothing special, simply the standards against which other treatments are compared. They do not have to be defined as 'zero input', 'farmer's practice' or similar, but will be chosen depending on the objectives.
- *Factorial treatment structure*: this can arise naturally from the hypotheses or objectives, but can also be used to test several unrelated hypotheses within one trial more efficiently than with separate trials.
- *Quantitative levels*: when treatments involve different quantities of something, then the actual levels required may not be defined by the objectives, but guidelines on choosing them are available.

Mead (1988) gives a thorough practical discussion of statistical issues in choice of treatments.

Defining suitable control treatments should not be difficult if the objectives are clear, but there can be conflicts if the trial has multiple objectives. Commonly, farming system and biophysical objectives conflict and in some cases (at least) two controls are needed. Examples are a biophysical control for process-oriented research and a farming system control for applied studies, or a forestry and an agricultural control because both of these represent well established alternative land uses (Dupraz, 1999). The concept of controls is ill-defined and it is clearer to simply consider the contrasts needed to meet the objectives and then the treatments needed for the defined contrasts.

Quantitative treatments are common in experiments concerning agroforestry and soil fertility – for example, amounts of organic or inorganic input and density of trees. For these types of treatments the objective usually reduces to estimation of a response curve. Choosing treatments involves choosing the range of levels (minimum and maximum), the number of different levels, the spacing between the levels and the degree of replication of each level. There is a mathematical theory of design applicable where a smooth response curve is expected, from which the following general rules emerge.

- Make the range as wide as feasible. Make sure the highest levels are well past any optimum.
- Limit the number of different levels; estimating a straight line needs just two. A third will allow its straightness to be checked. More complex curves may need four or five levels but it is very rare to find an example that needs more different levels than this.
- Put levels closest together where you think the response will change fastest. In the absence of any other information use equally spaced levels.

When there are two or more quantitative treatments there are important alternatives to complete factorial sets of treatments.

Many agroforestry experiments involve treatments of distinct agricultural systems, not just relatively minor variations of the same system. This means it can be difficult to define the management of the differing plots in a way which does not lead to confounding of treatment effects with management variables. A simple example from Kenya involved screening seven tree species for their effect on intercropped maize. An eighth treatment of monocrop maize was included. As this was a phosphorus-deficient site, trees were given a starter dose of phosphorus fertilizer but the monocrop was not, resulting in confounding of effects of trees and phosphorus fertilization. The design is acceptable for comparison of systems, to be assessed perhaps on the basis of profitability, but biophysical data on, for example, phosphorus cycling or even crop yield are difficult to interpret. Dupraz (1999) gives other examples of this problem, including confounding effects of pest and weed control.

Experimental layout

Layout of a trial involves choice of locations, definition of the plots, characterization of the site, decisions on replication, choice of blocks and allocation of treatments to plots. Much of the theory and practice of all these developed for agricultural trials is relevant to agroforestry trials. There are many books on the topic. Dyke (1988) gives practical field advice. Mead (1988) covers the more statistical theory. Notes by Coe (1997b,c) and Stern and Adam (1997) are also relevant.

There are two important differences between typical soil fertility experiments involving annual crops and those with agroforestry treatments which arise because of the differing time and spatial scales involved.

- Trees may take several years to produce the effects of interest, so trials will usually be in the ground much longer than those with annual crops. If a trial of one growing season turns out to have been poorly designed or laid out then it can be repeated the following season. This is not true for an agroforestry trial in which design faults associated with the tree component may not be apparent until years after it was planted. Hence, it is worth investing resources in ensuring the layout is as sound as possible. In particular it is worth characterizing the site to identify heterogeneity, which can then be allowed for by a combination of blocking, avoidance and increased replication (see also Section 3.6). Another implication of the long time horizon of agroforestry experiments is that they will inevitably be used for

purposes other than those for which they were originally planned. It is therefore worth building some flexibility into the design. This means keeping treatments simple, using large plots that can be split later and including extra replicates that may be modified as needed (for example, by destructive harvest).

- Trees are typically larger than annual crops, which also has implications for the layout of experiments. Most important is the avoidance of interference between neighbouring plots. Above-ground interference occurs through such effects as shading, windbreak effects and lateral transport of tree litter by wind. These may not be easy to control but at least can be seen and perhaps avoided. Below-ground interference occurs as tree roots extend beyond their nominal plot and capture resources from surrounding areas, either from other plots or from outside the experiment. In many trials it is clear that this has resulted in biased treatment comparisons. If a plot with trees is adjacent to a crop-only plot then the tree plot may capture extra resources and the crop plot may be deprived of resources relative to a situation in which both are grown in very large plots, resulting in a biased treatment comparison. The options for avoiding the bias are either to leave very large border areas or to physically prevent interference.

It is difficult to judge the size of borders required in any situation; this clearly depends on the characteristics of the species involved, the site and the length of time the trees will grow. Van Noordwijk *et al.* (1996) discuss



Fig. 3.1. Lateral root from a *Sesbania sesban* fallow plot (on the right) grown into a crop plot (*Cajanus cajan*) in about 4 months.

factors affecting tree root growth in agroforestry systems and make the point that it is very difficult to give general guidelines for the lateral extent of roots. Certainly, in dry areas, extensions of over 40 m have been recorded. Figure 3.1 shows a lateral root of *Sesbania sesban* known to have extended over 6 m in 4 months.

Physical prevention means installation of below-ground barriers or regular root pruning between plots. Barriers are of limited value as roots can quickly grow under them and up into adjacent plots (Rao and Govidarajan, 1996). Pruning below ground is expensive as trenches have to be re-opened each time it is done, possibly four times a year. Trenches cannot usually be left unfilled between prunings because of their impact on water movement and the likelihood that they will behave similarly to solid barriers, with roots growing under them. A third alternative that has been suggested is to lay out trials so that plots are not adjacent. This will probably decrease the precision of the experiment but, more importantly, will not remove the small plot bias as trees will still be capturing resources from outside their nominal area. In fallow experiments it is common to use large plots with large borders for the fallow phase of an experiment, then to split the plots for further treatment factors during the cropping phase. Remember that the original borders may still have to be left as they may be untypical even after the trees have been removed.

An alternative approach recognizes that, within many farming systems, agroforestry will be used in small patches in a landscape. This landscape will be full of edges between agroforestry and other land uses, and the lateral interactions between them may be an important component of the way the system functions. Indeed, this agricultural landscape mosaic with trees has been defined as agroforestry (Leakey, 1996). The implication for research is that interactions should not be removed from trials but incorporated into the objectives (van Noordwijk, 1999). Designing trials with a system focus using this notion is feasible, though challenging. The problems are likely to be both in selecting useful objectives from the myriad of possible topics, and installing, managing and monitoring trials of the size required. The alternative is to measure lateral flows in simpler experiments and then attempt to model the landscape level effects of agroforestry systems.

The longer time scale and larger plots needed in typical agroforestry trials, compared with annual crops, can make trials with farmers difficult. Farmers have to commit relatively large areas of land for long periods. The areas required mean that often each farmer can only test a few treatments and the ideas of incomplete blocks are needed to find effective designs. The long time periods also mean that both drop-outs and modifications by farmers are common, resulting in more replicates being needed.

Number of replicates

The number of replicates required in an experiment is straightforward to estimate in theory, but can be difficult in practice as information required is unknown before the trial starts. Replication serves three purposes in an experiment:

- to allow estimation of the random variation, and hence of the precision of contrasts of interest;
- to increase the precision of estimating important quantities, the average of a larger sample being more precise than that of a smaller one; and
- as insurance against disasters that lead to loss of parts of the trial.

The number of replicates needed to satisfy each of these purposes has to be looked at.

The quality of the estimate of a variance depends on the degrees of freedom associated with it. For a simple randomized block design the variance relevant to estimation of treatment differences is the residual or error variance. The degrees of freedom in the residual line of the analysis of variance table indicates how well that variance will be estimated from the design. Experimenters should aim for residual degrees of freedom to be at least ten, though one or two less than this may be acceptable. Residual degrees of freedom less than six are hopeless. For more complex designs which have several different layers, such as split-plot or multisite designs, there is more than one variance relevant to estimation of treatment effects, and each has to have sufficient degrees of freedom. The researcher in these circumstances has to be able to produce an outline analysis of variance for the design and check that degrees of freedom for each relevant error line are adequate. Some software can produce these outline analyses, but any software can be forced to do so by putting in dummy data, as the degrees of freedom depend only on the design, not on the data values. In agroforestry trials it is not unusual for the degrees of freedom to be different for different variables. For example, a trial that compares monocrop maize with maize intercropped with trees has more plots with crop data than with tree data. The analyses of variance for tree and crop variables will, therefore, have different degrees of freedom, so both need checking.

Estimation of replication required for the second purpose, controlling precision, requires experimenters to have an idea of: (i) the size of relevant error variances likely to occur in their trial, and (ii) the precision they actually require. The first of these has to be estimated from previous experience with other trials of a similar type in a similar environment. Although every trial should be different from all predecessors, there are few experiments that are so novel that it is not possible to get some idea of the expected levels of variation. The variation may be expressed as a

variance (σ^2) or independently of the units of measurement as a coefficient of variation (cv), which is simply the standard deviation divided by the mean. The precision required is most usefully expressed as the standard error or width (w) of confidence interval needed for critical quantities. Alternatively, it may be expressed as the size of effect that the trial needs to detect as significant. Formulae for the number of replicates may then be deduced. For example, for an approximate 95% confidence interval of width w for the difference between two means in a randomized block design, the required number of replicates (r) is

$$r = 32\frac{\sigma^2}{w^2} \tag{3.1}$$

Software is available for producing formulae for other designs and other specifications of the problem (Thomas and Krebs, 1997).

For the third reason for replicating, insurance, it is harder to judge the number required. If an experiment has a short lifespan (say one season) then there is little justification for insurance, unless perhaps under farmer management where there is some uncertainty about the likelihood of individual farmers adhering to important aspects of a protocol. However, if a long-term trial is in a situation where it might be at risk (say from fire) then inclusion of a few extra replicates may be judged worthwhile, particularly given the other reasons for building some redundancy into the initial design, mentioned above.

The situation for the simplest on-farm trials, designed to estimate the mean difference between two treatments, is no different from that for on-station trials, and the same information is required to estimate sample sizes. Variation at all levels tends to be higher, so numbers of replicates needed are often large. The large plots needed in many agroforestry trials mean that it is often only feasible to have one replicate per farm, and farms should be treated as blocks. If it is only possible to have less than one replicate per farm then incomplete block designs are useful, with an implication for the number of replicates needed. The statistical principles are described well by Mead (1988), and software is available to help find suitable designs.

Many on-farm trials have more complex objectives than simply comparing two treatment means, but are concerned with performance of agroforestry in different farm and landscape situations. Very often there is a hierarchy of levels in the design from individual plants at the lowest level to communities or watersheds at a higher level. At each of these levels there are design questions to resolve concerning the number of units, the selection of those units and the treatments or interventions to be compared on those units (Table 3.1). The same ideas are needed for determining the number of replicates in these more complex designs as in simpler ones.

Table 3.1. Hierarchy of levels and questions to be answered in design of on-farm experiments.

Layer	How many?	Which ones?	Who decides?	What intervention?	What is measured?
Village					
Landscape position					
Farm					
Niche					
Plot					
Tree					

3.3 Fallow Experiments (*R. Coe*)

The principles underlying the design and layout of fallow experiments are no different from those for other experiments, the key to a sound design being very clear and exact objectives for the trial. However, fallow experiments can give rise to particular difficulties because of the potentially large number of factors to investigate, the potential for plot to plot interference and the fact that time may be involved as a treatment.

If the experiment is system focused, aimed at evaluating the effect of management factors, there are questions concerning fallow establishment, management, clearing, post-fallow cropping and starting a second fallow cycle. Techniques similar to those described in Tripp and Wooley (1989) can be used to prioritize factors for investigation. The idea is to choose criteria such as ease and cost of doing the research, the extent to which the factor is critical to adoption of the technology, the length of time the research will take and the chance that it is successful. The various factors that could be investigated are then scored on each of the criteria to produce a ranking. The number of treatment combinations can sometimes be reduced by assumptions such as, for a given location and species, that

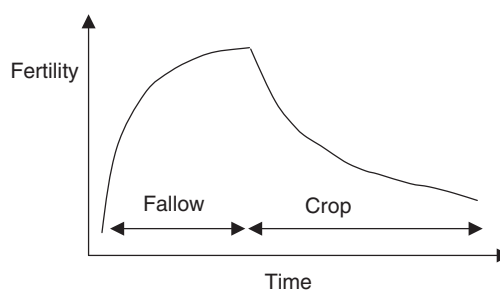


Fig. 3.2. Schema of soil fertility development during cropping and fallow cycles.

Table 3.2. Design of a fallow experiment comparing fallow lengths of one, two and three seasons.

	Season			
Treatment	1	2	3	4
1	F	C		
2	F	F	C	
3	F	F	F	C

F, fallow; C, crop.

there is a direct relationship between the fertility effect of the fallow and the fallow biomass (Mafongoya and Dzowela, 1999). When the trial has a process focus, the process objectives will determine treatments. Note in particular that species for fallows can often be chosen in a way that gives the trial much more value than a simple comparison of a range of likely species. An example is the comparison of four species deliberately chosen to evaluate the two characteristics of deep-rooted vs. shallow-rooted and nitrogen fixing vs. non-fixing. The control treatments might be a natural fallow, continuous cropping, a herbaceous fallow or even bare ground (Hartemink *et al.*, 1996), the choice, of course, depending on the exact objectives (see Section 3.2).

Much applied research on fallows is concerned with factors associated with the rate at which they restore fertility and the rate at which crops reduce it – the shape and slopes of the curves in Fig. 3.2.

Investigation of fertility recovery during the fallow phase requires comparison of fallows of different lengths. A design to look at fallows of one, two and three seasons may involve treatments as shown in Table 3.2.

The crops in seasons 2, 3 and 4 will reflect the changes in soil fertility following one, two and three seasons of fallow. However, the expected pattern (Fig. 3.2) will be confounded with the season-to-season fluctuations in weather and disease patterns. For this reason the phased entry design of Table 3.3 is preferable.

Table 3.3. Design of a fallow experiment comparing fallow lengths of one, two and three seasons (version 2).

	Season			
Treatment	1	2	3	4
1	*	*	F	C
2	*	F	F	C
3	F	F	F	C

F, fallow; C, crop.

Now the effects of differing fallow lengths are assessed by comparison within a single season. However, the design raises the two following questions.

- What should be put in the cells marked * ? At the start of the first season the whole site is presumably in the state required for the experiment. To avoid confounding fallow length with other factors we need the soil in treatment 2 plots to be the same as this at the start of the second season, and those of treatment 1 plots at the start of the third season. There is no practice that can guarantee this. If the trial is concerned with farming systems then it is common for the initial condition to be that state of degradation in which a farmer would abandon cropping and start a fallow phase. It is often assumed that one or two further seasons of cropping on these degraded plots will not substantially change the soil further, so the design used is as in Table 3.4.
- The designs in Tables 3.3 and 3.4 are appropriate if the seasonal effects (such as weather) apply to the crop but not the fallow. Although it is likely that in general a crop will be more sensitive to seasonal differences than a fallow, this need not be the case. Suppose the third season was a very good one for fallow establishment, but that the second season was not. This difference may be reflected in the following crops and be confounded with the comparison between the two seasons. A way around this is to repeat the sequences in more than one season (Table 3.5). It is clear that this design can quickly grow to something unmanageable, both because of the number of plots and the time involved. Of course there is no guarantee that the seasons sampled with just one repeat of the sequences will be sufficient. Sites are sometimes substituted for seasons, putting treatments 1, 2 and 3 down simultaneously in a number of locations.

When looking at the cropping phase of the sequence there are similar considerations. If we want to measure fertility decline over, say, three

Table 3.4. Design of a fallow experiment comparing fallow lengths of one, two and three seasons (version 3).

	Season				
Treatment	0	1	2	3	4
1	D	D	D	F	C
2	D	D	F	F	C
3	D	F	F	F	C

F, fallow; C, crop; D, crop on degraded soil.

Table 3.5. Design of a fallow experiment comparing fallow lengths of one, two and three seasons, repeated through two different seasons.

	Season					
Treatment	0	1	2	3	4	5
1	D	D	D	F	C	
2	D	D	F	F	C	
3	D	F	F	F	C	
4	D	D	D	D	F	C
5	D	D	D	F	F	C
6	D	D	F	F	F	C

F, fallow; C, crop; D, crop on degraded soil.

seasons of cropping following a fallow, the comparisons require a design as in Table 3.6. In the fourth season the first, second and third crops following a 1 year fallow are compared.

It is clear that the requirements for comparing differing cropping and fallow periods are mutually incompatible. It is not possible to both start and end different treatment sequences at the same time if their lengths differ. For this reason compromise is necessary, the usual solution being to hope that fallows are less sensitive to seasonal variation than crops. The fallow plots can be measured to find out whether growth is in fact similar in different seasons (survival and biomass at the end of the first season of growth would be appropriate for this purpose), leaving open the question of what to do if this turns out not to be so. A typical design to study the decline in fertility following fallows with durations of one and two seasons uses treatments 1–6 in Table 3.7.

An alternative approach uses the idea that it is possible to define a control treatment (perhaps fully fertilized) that responds to weather and other season-specific factors but is constant with respect to soil fertility. A trial to look at decline in fertility following fallow might then use just treatments 3, 6 and 7 from Table 3.7. Crop yield of treatments 3 and 6

Table 3.6. Design of a fallow experiment comparing cropping cycles of one, two and three seasons.

	Season				
Treatment	0	1	2	3	4
1	D	D	D	F	C
2	D	D	F	C	C
3	D	F	C	C	C

F, fallow; C, crop; D, crop on degraded soil.

Table 3.7. Design of a fallow experiment comparing cropping cycles of one, two and three seasons following one or two seasons of fallowing.

	Season					
Treatment	0	1	2	3	4	5
1	D	D	D	D	F	C
2	D	D	D	F	C	C
3	D	D	F	C	C	C
4	D	D	D	F	F	C
5	D	D	F	F	C	C
6	D	F	F	C	C	C
7	D	Cont	Cont	Cont	Cont	Cont

F, fallow; C, crop; D, crop on degraded soil; Cont, control.

during seasons 3, 4 and 5 would be assessed by measuring them relative to those in treatment 7. The approach depends on the assumption (untestable with the design) that there is a scale on which there is no treatment × season interaction, which seems unlikely, particularly when considering variables other than crop yield. Note that if treatments 1 to 7 of Table 3.7 are used then the value of this approach can be assessed by comparing the trends derived from the time series with those measured in season 5.

3.4 Measurements and Sampling Designs (*G. Schroth, R. Coe*)

Much of this book is concerned with measurement and there are many other sources with practical information both on what to measure and how to measure it. When planning experiments, it is worth thinking of the following three categories of measurements.

- Measurements that are needed to characterize the site in which the trial takes place, but do not vary between treatments. These include, for example, soil type and rainfall.
- Responses specified by the objectives, which might be soil nitrogen and plant growth.
- Measurements needed to understand variability in the key responses, which might be soil depth or texture and are often forgotten. They can be specified once the nature of the variability becomes apparent and field observation suggests some possible causes.

A special case of the first type of measurements are those that are specifically made to show that basic site characteristics do not vary significantly between treatments. This is especially important when proper

replication of treatments is not possible, because an experiment has not been established as such, but is rather a comparison of plots that have been under different vegetation cover or land use for a certain time (such as comparing agroforestry, conventional agriculture and natural forest). Such 'unplanned' comparisons often provide information that planned experiments cannot yield for practical reasons, especially if long time periods under a certain land use are needed. However, they always suffer from the difficulty of establishing whether the initial soil conditions were similar among sites. Sanchez (1987) suggested particle size distribution in the profile as the principal criterion for checking the comparability of sites.

Nearly all measurements in experiments require sampling of some sort and strategies for sampling soils are discussed in detail here. There are several recent descriptions of soil sampling procedures for agricultural (James and Wells, 1990) and environmental purposes (Crépin and Johnson, 1993). When adapting such recommendations for agroforestry situations, it is essential to be sure about the aim of the study, as this will affect decisions about the sampling plan as well as sampling depth and timing. There are two key aspects to be clear about.

- The level at which replication is needed to estimate precision. As an example consider plots of an experiment to be sampled to determine differences in soil properties under different treatments. Although several samples may be taken in each plot, it is not usually necessary to estimate the precision of values for each plot. Instead we need values from replicate plots to estimate precision of differences between treatment means.
- Whether it is necessary to compare different parts of the plot or field. In many agriculture or forestry experiments we do not expect different parts of a plot or field to vary systematically – one point is equivalent to any other point. This is not the case in typical agroforestry plots. Tree effects will not be uniform across a plot; for example, the soil properties under a hedgerow might be expected to differ from those under the crop between two hedgerows. The sampling used will depend on whether assessing this difference is required to meet the objectives.

Composite samples and bulking

In most cases, the objective of a sampling programme is to obtain average values of certain soil characteristics for a given area. In this case, the soil from different sampling points is bulked, mixed and a subsample taken for analysis. If this is done, then the variation between repeated subsamples gives no information about variation in the field.

In some cases it is necessary to take measurements on individual samples collected in the field. An example is the estimation of the precision of a plot average, usually the standard error of a mean of several samples in the plot. This information is useful for optimizing sampling schemes during preliminary studies but is of no use for the comparison of experimental treatments in different plots. Estimating precision is straightforward if the samples were collected in a random pattern. Systematic sampling does not allow precision to be estimated in a simple way, but estimates are available through the use of geostatistical methods to analyse the spatial patterns of soil properties in a plot (see Section 3.6).

Sampling uniform areas

An area to be sampled (a field or experimental plot) that is uniform, with no known or predicted patterns of variation across it, is unusual in agroforestry research. Possible examples for uniform areas are the crop-only control in an experiment, agricultural plots following a homogeneous fallow and perhaps very dense and homogeneous fallows themselves.

The sampling scheme determines where samples will be located in the area. Two schemes for this situation are *random* and *systematic*. Random sampling ideally means that the coordinates for each sample location are selected using random numbers, but in practice the samples are usually collected on a zigzag path across the plot (James and Wells, 1990). In systematic sampling, the sample sites are selected in a systematic way, typically using a grid pattern. For example, a 20 m × 20 m plot could be sampled by locating 16 points in a square 5 m × 5 m grid, starting 2.5 m from the edge of the plot.

Random sampling has the advantage that it is, in theory, unbiased, as no part of the plot is favoured. However, the practical application of random sampling can be biased as data collectors tend to avoid points they feel do not look 'typical'. Systematic sampling has the advantage that it is unbiased except in the case of the grid points coinciding with some pattern in the field. The example above would clearly be inappropriate if trees were also planted on a 5 m × 5 m grid. The practical application of systematic sampling is simpler than random sampling because the same sample positions can be used in every plot and the subjectivity found in practical random sampling is removed. Systematic sampling will nearly always give estimates of higher precision than random sampling because the sampling points are more evenly spread through the area of interest (Webster and Oliver, 1990).

Sampling non-uniform areas

Most areas to be sampled vary in a known or predictable way. The patterns of variation may be due to factors such as slope, soil depth or weed distribution. In agroforestry plots, pronounced variation of soil characteristics, litter and root distribution is often caused by the spatial arrangement of different trees and crops, and a common problem is how to obtain a sample that is representative for the whole plot in this situation. A useful approach to this problem is to identify the smallest representative unit (SRU), which is the smallest spatial element of which the plot (or system) is composed. For example, in a planted fallow with trees at 2 m × 2 m spacing, the SRU would be a 1 m × 1 m area with a tree in one corner (Fig. 3.3). In a system with trees planted in rows between crop fields with 5 m spacing between trees in the rows and 20 m between rows, the SRU would be a 2.5 m × 10 m strip perpendicular to a tree row with a tree in one corner if the direction from the tree row is not considered important (SRU 1 in Fig. 3.4), or a 2.5 × 20 m strip if there are directional effects of slope or prevailing wind that need to be taken into consideration (SRU 2 in Fig. 3.4). Even in a pure crop field the SRU concept may be useful, for example, when a field has been ridged, when a crop is still small and does not occupy the soil homogeneously or in most cases when root systems and related soil characteristics are studied.

To be representative for the whole plot or system, a sample would have to be representative for one or preferably more SRUs in a plot. To obtain a representative sample of litter in an agroforestry plot, an efficient strategy can be to collect the whole litter in one or more SRUs per plot, then homogenize and subsample. For measuring coarse root biomass, the excavation of the soil of one or more SRUs per plot to a certain depth may

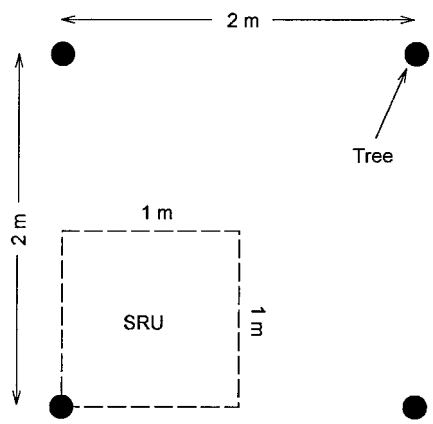


Fig. 3.3. Smallest representative unit (SRU) for sampling an area with trees planted at a spacing of 2 m × 2 m.

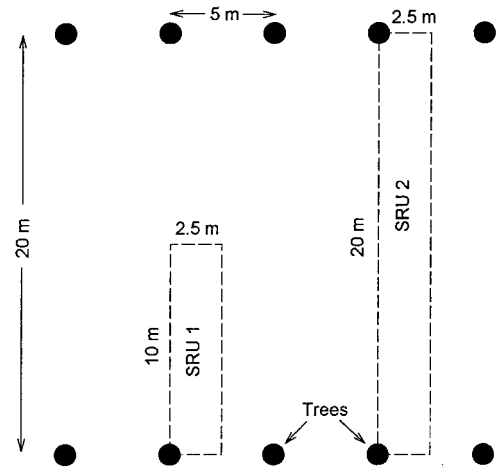


Fig. 3.4. Two options for the smallest representative unit (SRU) for sampling a crop field between rows of trees that are planted at 5 m × 20 m spacing. The choice between the two SRUs depends on whether a significant influence of the direction from the tree rows on the variables under study is expected (SRU 2) or not expected (SRU 1).

be necessary. For soil or fine root samples, the individual sampling points would have to cover one or more SRUs in a plot in a representative grid or other pattern.

In many cases, the precision of sampling can be increased, and additional information on spatial structures obtained, by *stratification*. This simply means dividing the plot into regions thought to be homogeneous, and sampling each of these strata. Within each stratum, either a random or a systematic sampling plan can be used. For example, in a pasture or crop field with scattered trees, the strata to be sampled separately could be: (i) the area influenced by the shade and litter of a certain tree species, and (ii) the area not directly influenced by the tree. In a hedgerow intercropping experiment, the plot could be subdivided into: (i) the area under the tree rows, and (ii) the area between the tree rows that is tilled, cropped and receives tree mulch. This cropped area could present gradients of nutrient availability caused by decreasing nutrient uptake by the trees and/or increasing nutrient uptake by the crops with increasing tree distance. In this situation, separate sampling of the soil under the trees and crops combined with systematic sampling within the cropped area (e.g. every 25 cm on a line crossing the area from one hedgerow to the other) ensures representative inclusion of soil from all tree distances. Stratified systematic sampling plans are usually the method of choice for root studies in agroforestry to allow for small-scale patterns in root mass and density around individual trees or tree rows.

When a stratified plan is used but interest is in the whole plot, care must be taken to weight the observations from each stratum appropriately (Rao and Coe, 1991). The following sampling design for a 1 m × 1 m tree plantation was used by Jama *et al.* (1998). The plot is divided into three strata according to how far each point is from the nearest tree. The strata of 0–0.28 m, 0.28–0.48 m and 0.48–0.7 m from a tree represent 25, 50 and 25%, respectively, of the area (Fig. 3.5). The choices now are: (i) sample each stratum, measure the soil property of interest for each and average the results using weights of 0.25, 0.5 and 0.25, respectively; or (ii) create a representative bulk sample from each stratum in the ratio 1:2:1. A sampling scheme with five instead of three tree distances for the same situation has been used by Mekonnen *et al.* (1999).

Pronounced spatial patterns of soil fertility within a plot, which have to be taken into account when defining sampling schemes, result not only from the presence of different tree and crop species in a system, but also from standard agricultural practices such as fertilizer placement or certain tillage methods. Tree crops are commonly fertilized non-uniformly, for example, in bands along coffee rows (on sloping land on the upper side of the row) or in a circle around the stem of larger, more widely spaced trees (as is common for citrus, oil palm and other tree crops). The area where the fertilizer is applied is often kept free from ground vegetation to reduce nutrient uptake by weeds or cover crops. Over the years, this

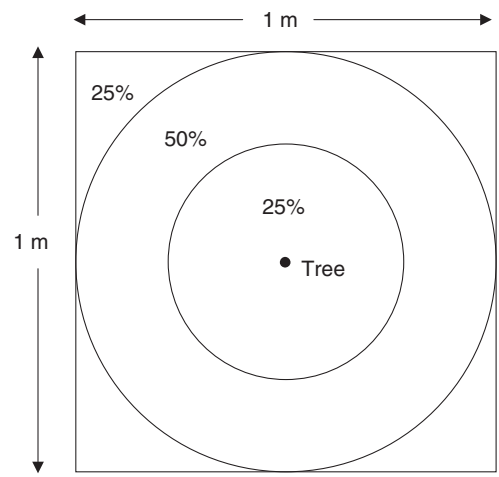


Fig. 3.5. Schematic diagram illustrating the subdivision of a plot with trees planted at 1 m × 1 m spacing into three sampling strata (reproduced with permission from Jama *et al.,* 1998). Note that the 1 m^2 square around the tree contains four smallest representative units and that for most research objectives it is better to collect one or two samples per distance per tree from several trees in a plot than many samples from a single tree.

practice can lead to pronounced differences in chemical soil characteristics between fertilized (and weeded) and unfertilized (vegetation-covered) areas in a plot. For example, available soil phosphorus (Mehlich 3) at 0–10 cm soil depth in an Amazonian oil palm (*Elaeis guineensis*) plantation was 165 mg kg^{-1} at 1 m stem distance, but only 4 mg kg^{-1} at 2.5 m stem distance (Schroth *et al.*, 2000a). In such situations, it is best to sample and analyse the fertilized and the unfertilized soil separately and to complement the analysis with a root distribution study to obtain an idea of the relative importance of these soils of differing fertility for plant nutrition. This example also illustrates how sequential samplings of the same plot could erroneously detect changes in soil fertility by collecting samples at slightly different positions if spatial fertility patterns are not taken into account.

Tillage practices that lead to pronounced spatial fertility patterns within a field include ridging, a common system not only on sloping land, but also on level areas, for example in West Africa. With a specially designed plough or a hoe, a strip of soil is turned and moved sideways, where it covers a soil strip of similar width and forms the ridge. Topsoil and weeds are concentrated in the ridges, on which the crop is sown. Microerosion progressively takes fine materials from the ridges into the furrows during the growing season. If sample collection before the ridging is not possible, sampling halfway up the ridges has been recommended (James and Wells, 1990), but it is often better to collect and analyse two separate samples from the ridges and the furrows, especially if different crops are grown in these two positions.

Comparing parts of a plot

In agroforestry experiments, plots are not only subdivided to avoid problems with sampling heterogeneous areas. In many cases, the analysis of the spatial patterns of soil properties that are caused by the different plant species is of considerable interest itself, as it may provide information on the different effects of trees and crops on the soil and so on tree–crop interactions. Examples would be systematic changes in soil nitrogen or soil water content with increasing tree distance in a crop field. Figure 3.6 shows a plot with a single row of trees down the centre with crops on either side (as would be appropriate for a shelterbelt). To measure the spatial variation of soil properties caused by the trees, samples have to be collected at different distances from the tree line. The principles are the same as for choosing quantitative levels of a treatment factor (see Section 3.2). If a simple response to distance is to be estimated, then it is better to use many samples at a few distances rather than few samples at each of many different distances. Typically, measurements will be taken in or close to the tree row (maximum tree effect), at a large distance (no tree effect) and

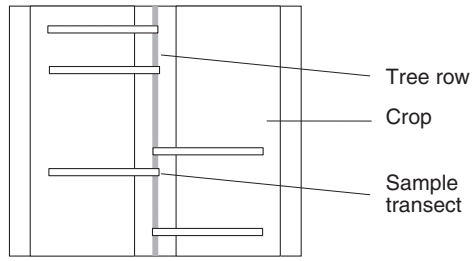


Fig. 3.6. Sampling scheme in transects in a plot with a single tree row and crops.

at one or two intermediate distances. Soil properties usually change most rapidly close to the trees, and it is thus advisable to collect samples at smaller distance intervals here and increase the intervals with increasing tree distance. Precision for comparing distances will be maximized if the samples fall in a number of transects, as in Fig. 3.6.

In certain cases, measuring a representative value for a whole plot or system may not be the main objective of a sampling strategy. It may be more important, for example, to detect extreme values within a plot. When tree species that differ in their effects on the soil are mixed in a complex system, such as a forest garden, it would be expected that the effect of each tree is most pronounced close to the tree and decreases with increasing distance from the tree, where it also overlaps increasingly with the effects of neighbouring trees. In this situation, the most sensitive sampling strategy for the detection of specific tree effects on soil fertility is the collection of soil samples taken from very close to individual trees of the different tree species for comparison with controls taken at points at a maximum distance from all trees, which are assumed to have the smallest influence from trees possible given the configuration of the system. The resulting single-tree effects on soil fertility cannot be quantitatively scaled up to the whole plot unless extensive sampling is undertaken to characterize the gradients and geostatistical techniques are employed (see Section 3.6), but they do give valuable qualitative information concerning the way a certain species and its management might influence soil conditions, which is often of more immediate practical use than quantitative mean data per hectare (see Schroth *et al.*, 2001b, for a review of agroforestry applications). In parklands and similar systems with naturally regenerated trees in irregular arrangements, care must be taken in the interpretation of observed fertility differences between tree and no-tree positions, as the trees may have established themselves on fertile spots, such as termite mounds or depressions receiving run-on water and eroded nutrients. The possibility of such initial fertility differences between positions may be excluded when the trees have been planted at a regular spacing, as in tree crop plantations.

Sampling depth

Soil sampling for agricultural purposes often includes only the topsoil, that is, the plough layer or a shallow surface horizon in no-till fields (James and Wells, 1990). In forestry, deeper sampling sometimes gives better correlations with plant growth or fertilizer response, for example 60 cm for phosphorus and 150 cm for potassium in different pine stands as reported by Comerford *et al.* (1984). For nitrate, sampling to 120 or even 180 cm depth has been recommended even for annual crops (Comerford *et al.*, 1984; James and Wells, 1990).

In agroforestry, sampling of the top 10 or 20 cm of soil may sometimes be adequate when treatments that are unlikely to affect the subsoil are being studied, such as the short-term effects of mulching, but it is inadequate for studies of competition and complementarity of nutrient use between different species. As a rule, the decision about sampling depth should be based on an at least qualitative analysis of the vertical root distribution of the relevant plant species (see Section 12.2). If this analysis reveals that one species (the crop) has relevant amounts of roots to 60 cm soil depth and the other species (the tree) to 100 cm depth, it is important to collect a separate sample from the 60–100 cm depth as the water and nutrients here may contribute to complementarity between the species (see Box 5.2, p. 100). If the tree penetrates a hardened subsoil horizon but the crop does not, the hardened horizon and underlying horizons should be included in the sampling, because increased access to the subsoil for the crop following root channels of the tree could be an important benefit of the association. If nutrient recycling from the subsoil is to be assessed, a preliminary evaluation of the nutrient distribution in the soil profile is useful.

It is common practice to take fewer samples from depth than from surface layers to reduce the sampling effort. However, few samples will lead to imprecise estimates, unless the variability of the investigated variable also decreases in the subsoil.

Sampling time and frequency

Certain soil characteristics change both during the year and between years, and sampling time has thus to be selected carefully in relation to the objectives of the study. Seasonal fluctuations have been reported for labile fractions of carbon, nitrogen and phosphorus as well as microbial soil characteristics in response to moisture changes, crop phenology and management (Conti *et al.*, 1992; Mazzarino *et al.*, 1993; Campo *et al.*, 1998). Soil test readings for phosphorus and potassium probably decrease during the cropping season, whereas soil acidity increases in acid, but not in alkaline soils (James and Wells, 1990). In long-term studies, samples

repeated in different years should be collected at the same time of the year relative to weather and crop development to be fully comparable (e.g. '2 weeks after crop emergence' or 'as soon as 50 mm cumulative rain has fallen after 1 September'). Short-term fluctuations of nutrient availability in response to management measures such as fertilization and mulching can also be studied by collecting and analysing the soil solution at appropriate intervals (see Section 7.2).

3.5 Analysing the Data (*R. Coe*)

There are many resources that describe standard statistical procedures for analysing data from experiments. There are, however, few books that actually describe the *process* of analysing data from field trials, so this is discussed briefly here.

The first step is to define objectives for analysis. The difficulty many scientists face with statistical analysis originates in part from the analysis objectives not being carefully thought through. Defining the objectives of analysis will involve:

- identifying the exact comparisons to be made or relationships to be estimated;
- determining the exact data that are needed to make the comparisons (for example, are comparisons of yield needed each season or totalled over all seasons?); and
- designing the tables and graphs that will be used to present the results.

The objectives of analysis are determined by the objectives of the trial. However, the analysis objectives are distinct from trial objectives in that:

- the objectives of the trial may well have been stated in a rather vague way;
- new objectives will have been developed as the trial progresses, resulting from observations made;
- the objectives set out in the original protocol may well have other, unstated, objectives added if these can usefully be met with the data available;
- it might not be possible to meet all the original objectives of the trial, either because the trial design does not allow this, or because something unexpected has happened to prevent it; and
- the objectives of the analysis will evolve as the analysis proceeds.

The second step requires preparation of the data. Almost always there is considerable work to do to turn raw field records into data files suitable for statistical analysis. Conversions and calibrations have to be completed. New variables have to be constructed. Data have to be summarized to the

appropriate space and time scale, and so on. The ease with which this is done depends largely on how the original records were organized. Statistical Services Centre (2000) is a good guide to optimizing this.

The third step is exploratory analysis of the data that aims at finding summary tables and graphs that meet the objectives. Well-constructed tables and graphs will make the patterns and relationships in the data clear and should make tentative conclusions obvious. The other aim of exploratory analysis is to reveal any unexpected observations or patterns in the data that might impact either on the conclusions or the way the analysis proceeds.

Next comes the confirmatory analysis. This is where formal statistical techniques are relevant and is the step widely described in books and taught in courses. The formal analysis aims to:

- confirm that the patterns and relationships noted in the exploratory analysis are consistent with real or repeatable effects, and are unlikely to be due to noise;
- estimate the precision of important quantities emerging from the analysis; and
- increase the precision of these estimates by finding parsimonious statistical models which describe the data, do not contain any unimportant terms and manage to explain much of the variation.

The final step involves interpretation and reporting. Iteration between the various steps of analysis is nearly always needed.

Data from agroforestry-fertility field experiments are not inherently different from data from other trials, so these general methods usually apply. However, there are some characteristics of data from agroforestry trials which can make it confusing to apply general methods.

Multiple outputs

In system trials aimed at evaluating the productivity of alternative systems there will often be multiple products (tree and crop products, for example). The options for analysing these are to look at them separately, to investigate the relationship between them and to combine them into a single index of production. The range of methods developed for analysis of intercropping trials are appropriate (Federer, 1993, 1998).

Measurements repeated in time

The time scale of agroforestry experiments means that measurements will almost inevitably be taken repeatedly on the same variable at several times.

These may be within one season, for example to look at nitrogen dynamics during a cropping phase, or across seasons to look at long-term development of systems or response to different weather. The statistical problem associated with repeated measures arises from the fact that observations on the same units at different times cannot be expected to be independent, with correlations between observations likely to be larger the smaller the time interval between them. The most common statistical procedures, such as those based on analysis of variance (ANOVA) and regression, require independence of observations. A large group of techniques under the general heading of *repeated measures analysis* has been developed to handle this problem. These techniques range from simple and approximate adjustments of standard analyses to statistical models that describe the correlations in detail. They are described in many textbooks and appear in standard software. However they can be difficult to apply, particularly if the times of measurement are irregular or different for different treatments.

There is one approach which is simple to apply and produces analyses which are well tuned to meeting objectives. The idea is to turn the repeated measurements on each experimental unit into a single value for each unit, that value being chosen to represent a quantity required by the analysis objectives. The steps are as follows.

1. Explore the data (usually by plotting the results for each treatment against time) in order to identify a pattern or effect that is of interest and needs formal statistical analysis.
2. Choose a number that describes the effect of interest. Examples include:
   - the total biomass produced over 5 years;
   - the difference in biomass produced in a wet season and a dry season;
   - the change in soil nitrogen between crop planting and leaf stage 6 minus crop nitrogen uptake, representing nitrogen losses in the early part of a season;
   - the time until soil carbon reaches some threshold level, based on interpolation of annual measurements;
   - the rate parameter k of a decomposition curve fitted to biomass decomposition data.

   These examples show that the summary can be something as simple as a total or difference, or as complex as the parameters of a non-linear curve fitted to trends. The important point is that the number describes the phenomenon of interest.
3. Calculate the number for each experimental unit or plot of the original design and analyse for treatment differences using the ANOVA dictated by the design.

4. Repeat the procedure for as many different summary numbers as are needed to meet all the objectives.

Measurements repeated in space

Repeated measurements also occur in space, as different parts of a plot are measured with the purpose of comparing, but they do not represent separately randomized treatments. In many soils experiments, measurements are taken at different depths. Agroforestry experiments often have the added complication that different positions within a plot are not equivalent, for example different distances from a line of trees. The statistical problem associated with these repeated measures in space is identical to that posed by repeated measures in time – the observations from different parts of a plot cannot be expected to be independent. The approaches for dealing with the problem are also similar, though the statistical modelling of correlation structures in space can be rather harder than the equivalent in time. The simple approach based on summary quantities is also applicable to repeated measurements in space. For example, suppose tree and crop root length densities are measured at five different distances from a tree line and at eight different depths within each plot of an experiment. Depending on the exact objectives, examples of suitable summary values to be calculated for each plot and subjected to formal analysis might be:

1. Proportion of tree roots below the crop root layer;
2. Total tree roots per metre of tree line;
3. Root length density of crops in the zone 0–50 cm deep and less than 3 m horizontally from the tree line;
4. Parameters A, k and c in the model $A \exp(-k(h^2+cv^2)^{0.5})$ that describes the change in tree root length density with horizontal (h) and vertical (v) distance from the base of the trees.

It is likely that the summary chosen cannot be calculated in the same way for all treatments. Suppose the examples above come from a trial that compared four different tree species grown in a crop, together with a crop-only treatment with no trees, a total of five treatments. Summary (1) is simply not defined for the crop-only treatment. When analysing this variable there are only four treatments to consider. Summary (2) makes sense for the crop-only treatment but has the fixed value of zero. Since there is no uncertainty in this treatment mean, it is not correct to include these zeros in the analysis along with other values. When comparing a mean from a tree plot with that of the control we are comparing an uncertain non-zero mean with a known value of zero. For summary (3) we may well want to compare the value from tree plots with that from crop-

only plots. The value for the crop-only plot will be calculated from all measurements 0–50 cm deep in the plot, as different locations horizontally are all equivalent. The fact that the summary is calculated in different ways in plots of different treatments does not affect the formal analysis, apart perhaps from inducing some non-constant variance, which can be allowed for by suitable weighting.

Software for experimental design

No software will design experiments for you. However, there are several components of experimental design for which software can help. Software to help estimate the number of replicates or sample sizes needed is reviewed by Thomas and Krebs (1997). Their online paper also gives links to providers of commercial and free software.

Design of efficient incomplete block designs, neighbour designs and other advanced designs that allow for some site heterogeneity is assisted by the CycDesign software from the Commonwealth Scientific and Industrial Research Organisation (CSIRO), Australia (www.ffp.csiro.au/tigr/software/cycdesign). Similar facilities are available with the Genstat programme (www.nag.co.uk). There are many software products available aimed at helping to design industrial experiments. These are mainly concerned with selection of efficient treatment combinations from very large multifactor sets and have little application in agroforestry research.

Software for statistical analysis

There are hundreds of products to help in statistical analysis of data from experiments. The number around is now so large that there seems to be no systematic and reasonably complete list or directory to them. Internet sites of many statistical organizations contain links to software sites. One extensive listing that may be useful is found at www.stats.gla.ac.uk/cti/links_stats/software.html#packages. Many of the products listed here are accompanied by independent reviews. In addition to statistics packages there are facilities for certain statistical manipulations in many other types of software – spreadsheets, databases, even graphics software. With such a large choice it is difficult to make recommendations, though some general comments may be useful.

First, it is sensible to use a dedicated statistics software package for statistical calculations. An excellent article explaining the limitations of the spreadsheet Excel for statistics, for example, is found at www.rdg.ac.uk/ssc/dfid/booklets/topxfs.html. Similar points could be made about trying to do anything other than the simplest statistics with, say, database or graphics systems.

Secondly, there is no statistical software that meets all the requirements of any analyst. For example, S-Plus (www.splus.mathsoft.com) has many of the more recent developments in statistical methodology incorporated, but has a steep learning curve and is not easy for beginners to start using. Minitab (www.minitab.com), on the other hand, is ideally suited to beginners but may well not be able to analyse some of the more complex designs found in agroforestry research.

Generally you get what you pay for. It is unlikely that you will find free or shareware products that allow you to complete effective analyses. However, cost is not a guarantee of quality or appropriateness for analysis of experiments, and software which is sold as a general statistical analysis system may not be particularly suited to analysis of experiments as opposed to surveys, for example, SPSS (www.spss.com). Possibly the only freely available statistical software that does make a suitable introductory package for experimenters is INSTAT (www.rdg.ac.uk/ssc/instat/instat.html), which comes with extensive high quality documentation and examples.

SAS (www.sas.com) is often considered as the standard against which other statistical software is judged. It certainly has very extensive facilities and is widely used. Because of this, analyses using SAS are described in many books. However, there are some disadvantages. It can be difficult to carry out some non-standard analyses or manipulations that are conceptually straightforward. More importantly, SAS has developed a system for maintaining, integrating and delivering information from a wide range of sources. The applications development and database facilities have become very advanced, but will probably not be exploited by the agroforestry researcher, who ends up having to pay for software that will not be used. The decision on whether or not to invest in SAS will generally be an organizational rather than an individual one.

Many less-common analyses will require special software. Software for spatial statistics is reviewed in Section 3.6. Other examples of possible relevance are MIM (www.hypergraph.dk) used to unravel complex multivariate dependencies, or PC-ORD (www.ptinet.net/~mjm) for analysis of species composition data.

Given the great choice in software available and the range of tasks the analyst of agroforestry trials has to carry out, the only strategy that can be recommended is to acquire one of the well known and general statistical analysis systems and become proficient at using it, but recognize that additional software may be needed for particular jobs. The choice of which main package to use will often depend on who else is using it and what other tasks, beyond analysis of agroforestry experiments, it will be used for. An example of software which allows straightforward analysis to be completed easily, leads naturally to more complex analyses and overall maximizes the chance of doing a sound analysis is GENSTAT (www.nag.co.uk).

3.6 Spatial Structure and Its Analysis (*B. Huwe*)

There are several aspects to problems associated with spatial heterogeneity or variability in agricultural and agroforestry systems. On the one hand, field variability is regarded as a major obstacle in identifying parameter impacts or relationships between system components. High variability obliges the experimenter to increase the number of replicates in the experimental design or to subdivide the study area into several, separately sampled strata, which increases the number of samples and the complexity of the data analysis (see Section 3.4). On the other hand, the spatial variabilities of soil, vegetation and other properties are important system characteristics and thus of interest themselves. For example, the detection of spatial correlation between the distribution of plant species or their root systems and soil properties may be a first step in the analysis of plant–soil interactions (Adderley *et al.*, 1997; Mekonnen *et al.*, 1999). Further, in many applications extreme values can be more important than mean values, such as leaching in macropore systems, influenced by tree roots or macrofauna (Beven, 1991), or denitrification in 'hot spots' such as local clay enrichments or soil aggregates with reduced aeration (Clemens *et al.*, 1999). Spatial patterns and probability distributions are essential in studies where uncertainty analyses are involved, such as the risk of yield failure in drought years as influenced by variable soil texture or soil depth in a landscape, groundwater pollution with pesticides or nitrate (Soutter and Pannatier, 1996; Wade *et al.*, 1996), or N_2O emissions into the atmosphere (Velthof *et al.*, 1996; Clemens *et al.*, 1999).

This section focuses mainly on geostatistical concepts, which means spatial analyses and predictions, and their potential applications in agroforestry research. Limitations of geostatistical methods are pointed out, and some alternatives are mentioned. For procedures that allow the simultaneous treatment of space–time systems, see Myers (1992) and Panesar (1998). A short and incomplete list of available software is given at the end of this section.

Potential use of spatial statistics

Geostatistics provides useful tools for spatial analyses, creation of optimized maps and simulation of spatial processes. For example, geostatistics can be used to obtain estimates of the total nitrogen accumulation in the soil under a legume fallow, or total phosphorus availability in the soil of an agroforestry plot with complex spatial patterns of fertilizer application and nutrient uptake by plants. When creating maps of chemical or physical properties of a field or landscape, information on the similarity of values from neighbouring samples and increasing dissimilarity of values with

increasing distance between the samples, the so-called autocorrelation structure for the area, can be used to calculate weighting factors which minimize the estimation error of the variables under study (Goovaerts, 1998). Geostatistical methods can also be used for the generation of optimized sampling schemes according to the requirements of the experiment, financial constraints or predefined local accuracy (Webster and Burgess, 1984), thereby reducing the experimental effort. Further, it is possible to identify optimal pixel sizes for process models (Wade *et al.*, 1996) or to generate parameter fields for the analysis of the transport of nutrients or pollutants in structured soils (Piehler and Huwe, 2000). Lopez and Arrue (1995) used geostatistics to optimize an incomplete block design for tillage experiments which proved to be considerably more efficient than the corresponding complete block design.

Although geostatistical methods have been widely used in the field of soil, agricultural and hydrological sciences at different scales (Oliver, 1992; Schiffer, 1992; Webster and Boag, 1992; Huwe, 1993; Hoosbeck and Bouma, 1998), not much work on spatial analysis has been reported so far in the field of agroforestry. However, potential benefits for agroforestry studies are demonstrated by applications in forest and agricultural ecosystems (Sylla *et al.*, 1996; Bragato and Primavera, 1998; Gorres *et al.*, 1998), orchards (Gottwald *et al.*, 1995), shrub–steppe ecosystems (Smith *et al.*, 1994; Halvorson *et al.*, 1995) and in soil surveys (Di *et al.*, 1989). The scale of application ranges from the microscale of soil pores (Grevers and Jong, 1994) through agricultural fields (Bragato and Primavera, 1998) and landscapes (Wade *et al.*, 1996) to regions of several thousand square kilometres (van Meirvenne *et al.*, 1996). Processes involved in the studies comprise tree growth (Meredieu *et al.*, 1996), crop yield (Wopereis *et al.*, 1996), water transport in soils (Mulla, 1988), evaporation (Lascano and Hatfield, 1992), carbon and nitrogen mineralization (Smith *et al.*, 1994), nitrous oxide fluxes (Velthof *et al.*, 1996) and pesticide transport to the groundwater (Soutter and Pannatier, 1996). As water and matter fluxes play an increasing role as indicators for sustainability and environmental compatibility, the aforementioned aspects of heterogeneity will be of increasing importance in future agroforestry studies. The epidemiology of plant diseases and even the spatial distribution of leaves and fruits in trees have also been studied by means of geostatistical methods (Chellerni *et al.*, 1988; Monestiez *et al.*, 1990; Orum *et al.*, 1999).

General principles of geostatistics

Spatial variability, or spatial patterns, can conceptually be divided into components which are caused by either deterministic or stochastic processes. Apparently stochastic behaviour of a system may be induced by

so-called hidden variables (i.e. variables that were not measured), or simply by the scale of measurements. For example, to predict nitrate leaching in the soil, we may use an average root length density and average hydraulic conductivity per soil layer as determined from a number of field measurements as input variables into a deterministic model. However, even in the best of cases the output of this model will not be correct for every single spot in the area, because not every spot has average properties with respect to root length density or hydraulic conductivity and, of course, we can never know the exact root length density and hydraulic conductivity at every spot in the soil. Thus, whereas part of the factors that influence nitrate fluxes are 'known' (i.e. have been described through measured, average values), another part will always remain unknown and will introduce unexplained (apparently stochastic) variability in the behaviour of the system. As mentioned before, this unexplained variability can be so large in certain cases that the model result will be completely wrong, simply because the behaviour of the system is not determined by average but rather by extreme values.

The identification, analysis and description of the deterministic system components belong to the field of deterministic, process-based modelling, whereas the analysis and modelling of stochastic patterns is done within the framework of the theory of stochastic processes, including geostatistical theory. Classical statistical methods which are generally used in agroforestry largely ignore spatial structures. Most tests assume independence of sampling points and, in addition, normally distributed variables or residuals. By ignoring spatial autocorrelation structures, an error is introduced into the analysis.

The fundamental backbone of geostatistics is the concept of regionalized variables, which is based on the work of engineers, mathematicians, physicists and biometricians in the early 1940s (Mathes and Ries, 1995). The theory in its present form was developed mainly by Matheron (1963). For original literature and more complete descriptions of geostatistical theory and applications see Journel and Huijbregts (1978), Journel (1989), Bárdossy (1992) and Webster and Oliver (2001).

The main idea behind geostatistics is the common observation in the field that values of variables often resemble each other more as the distance between sampling points decreases. With increasing distance, the spatial influence of neighbouring samples becomes smaller, and above a certain limit, the so-called range, variables are independent in a statistical sense. Measurements are treated as realization of an ergodic spatial stochastic process, which means that the characteristics of the stochastic pattern or process can be derived from this single spatial data set. For example, a single measurement of soil phosphorus in several sampling positions in a plot is enough to describe the stochastic pattern of phosphorus distribution in the plot. Another assumption of geostatistical theory is intrinsic (or weak)

stationarity, which means that for any two sampling points in the area, the difference in the measured value (e.g. soil phosphorus content) depends only on the distance between the two points (the lag), independently of where in the plot the two samples were collected. This assumption is often problematic, especially in areas with pronounced soil gradients or abrupt changes in soil properties, as will be discussed further below.

Procedure of geostatistical analysis

A basic component of geostatistical analysis is the semivariogram (Fig. 3.7). A semivariogram is a mathematical function that describes how two sampling points become more different with increasing spatial distance between them, until a certain distance when the points become independent from each other. The semivariogram describes the autocorrelation structure of the measured variable in the respective area. The mathematical formula of the semivariogram is given further below.

Geostatistical studies involve the following steps (Kitanidis, 1997):

- design of the sampling grid;
- data collection;
- analysis of spatial structure (determination of empirical semivariograms);

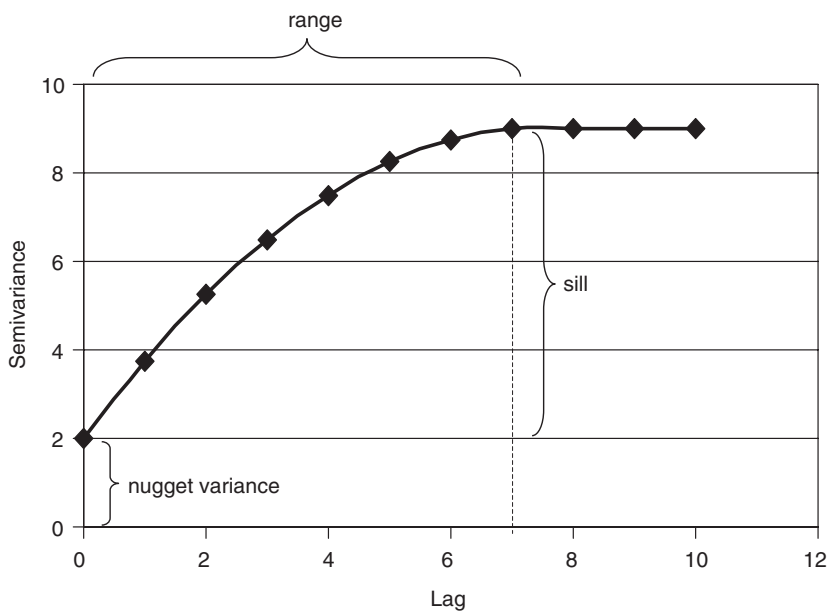


Fig. 3.7. Schematic semivariogram with a nugget variance of 2, a sill of 7 and a range of 7.

- selection of an appropriate semivariogram model;
- interpolating the grid by best, linear, unbiased estimations (BLUES); and
- interactive optimization of the semivariogram model using cross-validation techniques.

The design of the sampling grid (dimension, grid size, regular or irregular spacing) depends on problem-specific accuracy requirements and the spatial structure of the sampling area. One-dimensional grids (transects) are used for linear structures, such as gradients between linear tree plantings and adjacent fields. Two- and three-dimensional grids are used for maps, for example of nutrient distribution in a plot, or for the determination of parameter fields for process simulations, such as the transport of nutrients and pollutants in soil. Webster (1985) found that for isotropic semivariograms, i.e. in areas where soil properties are expected to change according to the same pattern in all directions, the best two-dimensional sampling scheme is a regular, equilateral triangular grid. For the estimation of mean values of square blocks, the best position of sampling points is in the centre of the blocks (Webster and Burgess, 1984). However, in practical studies it is often not possible to assure a regular grid. Further, in many cases it is advantageous to use a nested grid in order to determine the semivariogram for short sampling distances (Selles *et al.*, 1999) and to focus the accuracy of the calculated map on a predefined area of special interest (e.g. a sampling plot within a larger section of the landscape).

A qualitative explanation of the semivariogram has been given above. The mathematical formula of the semivariogram $\gamma(\vec{h})$ is given by

$$\gamma\left(\vec{h}\right) = \frac{1}{2N\left(\vec{h}\right)} \sum_{i=1}^{N\left(\vec{h}\right)} \left(z\left(\vec{x}i\right) - z\left(\vec{x}_i + \vec{h}\right)\right)^2 \tag{3.2}$$

where $z(\vec{x})$ denotes the measurement at location $\vec{x}$, and N is the number of pairs with a distance of $\vec{h}$. The arrow indicates vectors. In simple words, the semivariogram gives for every distance h between two data points a mean squared difference, obtained by averaging all pairs of data points with this distance in the data set. For a small h (samples collected close to each other), this difference will be relatively small, and for a larger h it will be greater. The use of vectors in the formula indicates that the semivariogram may not be the same in all directions, i.e. it may be anisotropic.

As illustrated in Fig. 3.7, typical semivariograms are characterized by: (i) a short-range variability, the nugget variance, which is determined by the closest sampling distance in the grid and is therefore to a certain extent an artefact; (ii) an asymptote equal to the background variance of the

variable, i.e. the variance between sampling points that are completely independent of each other at the scale of the study; and (iii) a maximum lag value that characterizes the range of spatial influence and is therefore called range. Points that are farther apart than this range are independent at the scale of the study. The sill is the difference between the background variance and the nugget variance. For irregular grids and anisotropic conditions, the differences are grouped into several distance and angle classes.

The experimental semivariogram is the basis for the selection of a theoretical model, which is then used for the analysis of spatial structures of the variable under study. Theoretical semivariogram models are mathematical functions that resemble the empirical semivariogram as closely as possible. Not all functions that would fit the data can be used as semivariogram models. If the model is to be used for spatial estimates or spatial simulations, it must be positively defined, a mathematical requirement that will not be discussed here (see Journel and Huijbregts, 1978). Unfortunately, it is not trivial to prove whether a given function fulfils this requirement or not. Custom models that are known to fulfil this criterion are the linear, spherical, exponential, logarithmic and Gaussian models. Other positively defined models are the quadratic, rational quadratic, power and wave models (Alfaro, 1980; Cressie, 1991; Pannatier, 1996; Kitanidis, 1997). Models for anisotropic conditions are also available. The field of variography is still in development.

A first estimate of semivariogram model parameters can be obtained by a least squares approach using the empirical semivariogram data. In a subsequent procedure, a number of different kriging techniques (see below) can be used to optimize the semivariogram and yield the best-possible estimated data. A criterion for the quality of the estimation is obtained by jack-knifing, a cross-validation procedure where each measured point is estimated from the neighbouring points and the estimated values are compared with the true values. The correct optimization algorithm for the semivariogram thus includes the whole estimation procedure, including the krige algorithm itself (Kitanidis, 1997), and not only the fitting of measured and estimated semivariogram data.

Point kriging

Kriging is a linear unbiased estimator for the variable under study. It is calculated as the weighted mean of the values of all sampling points or of the points in a user-defined local neighbourhood (which often ascertains local stationarity and thus avoids trend corrections, see below):

$$\hat{z} = \sum_i \lambda_i \cdot z_i \tag{3.3}$$

where z_i denotes the measured values, $\hat{z}$ the estimate at a user-defined location and λ_i the weighting factors calculated from the krige system. Kriging is also BLUES – the best, linear, unbiased estimator. Kriging, by definition, minimizes the estimation variance, which is the variance between the measured and the estimated data points. For each estimation on a location between the grid nodes, kriging involves the solution of a linear equation system, the krige system, that yields the weighting factors λ_i for the linear estimation procedure and a so-called Lagrange multiplier μ, which results from the consideration of the constraint $\sum_i \lambda_i = 1$. With the λs and μ it is possible to calculate the estimation variance for each estimated value and thus to determine confidence intervals. If the stochastic assumptions of the krige procedure are fulfilled, we may thus obtain not only maps with estimated (rather than mean) values, but in more or less the same step also maps that show the reliability of the estimated data, a source of information that is not provided by any other interpolation procedure.

Special kriging techniques

Point kriging, as described above, is the simplest kriging technique and for many applications in agricultural or environmental sciences it is sufficient. For example, maps of soil texture or pH can be generated with this technique. There are also several advanced kriging techniques for special data structures, quality aspects and project goals that are briefly introduced below.

Trends

A serious violation of the intrinsic model assumption of weak stationarity is given by a spatially non-constant expectation of the random variable under study, i.e. a trend. Trends may be caused by gradients in soil conditions in sloping areas, gradually changing geological or climatic site conditions, or land-use history. Common techniques to avoid the associated errors are the moving neighbourhood algorithm and the universal kriging approach. While the moving neighbourhood technique assumes local stationarity and thus restricts the grid points used for estimation to this neighbourhood, universal kriging assumes a predefined functional behaviour of the trend and eliminates this trend globally in an iterative algorithm (De Marsily, 1986; Deutsch and Journel, 1992). These techniques are available in some of the software packages listed at the end of this section.

Block kriging

Sometimes it may be desirable to estimate not point values but values that represent the average of the variable in a defined volume around the estimation point. Examples include the estimation of nutrient stocks or plant-available water in a given soil volume. The average variable is obtained by integrating over a volume V:

$$\bar{z} = \int_V z(\vec{x}) \mathrm{d}V \tag{3.4}$$

The estimation process is analogous to point kriging and differs mainly in the averaging of the semivariances in the system of linear equations. As a consequence of averaging, the estimation variances are typically much smaller than those obtained by point kriging (De Marsily, 1986; Deutsch and Journel, 1992). Gorres *et al.* (1998) used block kriging for the generation of maps of carbon mineralization, bulk densities and nematode densities in a forest and agricultural field.

Co-kriging

In most field studies, a number of parameters are measured at each sampling point rather than a single value. Many of these parameters may be intercorrelated as well as autocorrelated. Thus, taking into account the complete correlation structure should yield better estimation results compared to separate kriging of every single variable. Co-kriging was used by Halvorson *et al.* (1995) to analyse the spatial relations between resource islands in the soil and different plant species in a shrub–steppe ecosystem, and similar applications in savannas and parkland systems could be useful. As in simple kriging, co-kriging minimizes the estimation variance and provides its value for each location. Extensions to more than two correlated variables are possible (De Marsily, 1986).

Indicator kriging

This is a technique that may become particularly useful in environmental decision problems, such as when an environmental measure depends on the percentage of values above or below a given threshold in the model area (Akin and Siemes, 1988; Mulla and McBratney, 1999). Indicator kriging requires the transformation of the variables into indicator variables (values above a defined threshold are assigned a value of 1, values below the threshold are assigned a value of 0). Variography and kriging are conducted as described above. Kriging is carried out as simple point

kriging. Spatial averaging of the kriged indicator values yield the proportion of values of the original data set that are below the threshold. Halvorson *et al.* (1995) used this technique in the aforementioned study to determine the probability that certain combinations of variables occurred at unsampled locations.

Geostatistical simulation

Kriging is an interpolation algorithm that is optimal in the aforementioned sense. However, it generates more or less smooth maps that are in some ways unrealistic when compared with the real situation in the field. Unfortunately, this not only is of interest from an aesthetic point of view but may even yield incorrect results. This is always the case when extreme values are of importance. For example, transport of nitrate or pesticides in soil is often governed by preferential pathways, caused by macropore systems or spatial heterogeneity of soil hydraulic properties. Using simple similarity concepts together with geostatistical simulation, it is possible to generate heterogeneous parameter fields. Effects of this simulated heterogeneity for transport processes may then be calculated with numerical transport models (Piehler and Huwe, 2000). For example, a critical parameter of denitrification in soils is the soil water content. As kriging is a smoothing estimator, the percentage of water content events above a critical value, and consequently denitrification and the production of nitrous oxides, may be underestimated. Conditional geostatistical simulation provides a measure to generate more realistic maps that are compatible with the determined semivariogram. Furthermore, in the grid nodes the simulated values are identical to the measured values. Several algorithms for spatial simulation are available (e.g. the random coin method by Alfaro, 1980). A comprehensive overview with algorithms and FORTRAN-codes is given in Deutsch and Journel (1992).

Alternative methods and tools

Geostatistical methods have some serious drawbacks. Although normal distribution of the random variable is not explicitly required, it is recommended by some authors and may facilitate the interpretation of the maps of estimation variances. The applicability of geostatistics is restricted to situations where ergodicity may be assumed and the intrinsic hypothesis is valid. Thus, trends and structural discontinuities render the geostatistical analysis more difficult. Such situations may be caused by short-range, sharp gradients in a structured agroforestry system as well as by marked changes across a landscape (e.g. the boundary between

grassland and forest), and geostatistics may then not be the appropriate methodology.

Recent developments in mathematics and statistics provide algorithms and tools that also have potential for spatial statistics, although up to now they have not been studied and tested in full detail. Most of the methods have in common that they are robust and do not prescribe probability density functions like the bell-shaped Gaussian distribution. See Mathes and Ries (1995) for local gradient analysis, Batchelor (1998), Levine *et al.* (1996) and Liescheid *et al.* (1998) for artificial neural networks, and Woldt *et al.* (1992) for a geostatistical application of fuzzy set theory.

Geostatistical software

Some information is given below about software available in the public domain (PD) and commercially (®).

- GEO-EAS[PD] and GEOPACK[PD] are DOS-programs that provide basic functionality of variogram analysis, kriging and visualization ('rough and dirty'). The DOS-environment is not very user friendly and does not fit into modern Windows systems (www.hydroweb.com).
- SURFER® (Golden Software, 1999) is a contouring and surface mapping package that provides variogram analysis, variogram fitting and kriging, including block kriging and universal kriging (www.goldensoftware.com).
- GSLIB[PD] (Deutsch and Journel, 1992) is a most powerful collection of FORTRAN-programs that covers almost every aspect of geostatistics. The handbook is a very good overview of geostatistical theory. The user must be familiar with FORTRAN and have access to a FORTRAN compiler.
- SYSTAT® is a commercial statistical package that now includes geostatistical routines based on the GSLIB package and CART-Algorithms (www.spss.com/software/ science/SYSTAT).
- GS+® is a commercial geostatistical software package with a user friendly interface (www.gammadesign.com).
- DATA-ENGINE® provides algorithms for fuzzy control, artificial neural networks and combinations of both (www.mitgmbh.de).
- Stuttgart Neural Network Simulator (SNNSPD) is a very powerful artificial neural network (www-ra.informatik.uni-tuebingen.de/SNNS).
- VARIOWIN (Pannatier, 1996) is an easy to use software package for variogram analysis and visualization.

Chapter 4
Soil Organic Matter

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4.1 Synopsis

Soils contain a variety of organic materials, ranging from living roots, fauna and microbes, through dead tissues in various stages of decomposition, to relatively stable, dark-coloured transformation products with no discernible anatomical structures, the so-called humus (Jenkinson, 1988b). Following Jenkinson (1988b), the term soil organic matter includes all organic substances in soil, both living and dead, although the living organisms (the soil biomass) usually contribute less than 5% to the total soil organic matter. Certain terminologies do not include the biomass in soil organic matter, and some authors use the terms humus and soil organic matter synonymously, so care is necessary to avoid misunderstanding. In practical terms, analyses of soil organic matter are usually carried out on air-dried soil that was passed through a 2 mm sieve and will include all living and dead organic materials in the soil that are not removed by this procedure (Anderson and Ingram, 1993). In studies relating soil organic matter contents or fractions to soil aggregation, particles >2 mm may also be included in the analysis. The organic materials that lie on the soil surface, including naturally fallen litter, crop residues, mulch materials and their decomposition products, are normally sampled and analysed separately from the soil (see Chapter 6). Because decomposing roots have a function similar to that of above-ground litter in the replenishment of soil organic matter, they are sometimes called below-ground (or root) litter. This should not be confused with the term soil litter, which has been used for a density/particle size fraction of soil

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organic matter (see Section 4.3). A detailed discussion of the chemistry, formation and transformations of soil organic matter is provided by Stevenson and Cole (1999).

The organic carbon contents of terrestrial soils vary between <1% in many sandy soils and about 3.5% in grassland soils. Poorly drained soils may have much higher organic carbon contents (Stevenson and Cole, 1999). Contrary to widespread belief, the organic matter contents of tropical soils are not generally lower than those of temperate soils; in fact, they vary with vegetation (higher in forest than in savanna soils), climate (higher in mountain forests than lowland forests), soil texture (increasing with increasing clay and silt content of the soil), mineralogy (higher in volcanic soils due to the stabilizing effect of allophane on soil organic matter) and soil use (Feller *et al.*, 1991b; van Wambeke, 1992).

Role of soil organic matter in soil fertility

The organic matter content of a soil influences a wide range of soil properties and processes and, despite the fact that plants do not require organic matter as such for their growth and development, it is considered one of the most important components of soil fertility (Gregorich *et al.*, 1994). This is especially so in agriculture with low external inputs. In soils dominated by low-activity clays such as kaolinite, the cation exchange capacity, and thus the ability of the soil to retain nutrients against leaching in a plant-available form, depends strongly on its organic matter content. Soil organic matter may act either as a source or a temporary sink of nutrients such as nitrogen, phosphorus and sulphur (see Sections 5.2–5.4). Organic substances can increase the solubility of phosphorus in the soil and reduce phosphorus fixation by competing with phosphate ions for sorption sites such as oxides of iron and aluminium and clay minerals (especially allophane in volcanic soils). They can also complex aluminium, iron and calcium cations, which would otherwise form precipitates of low solubility with phosphate. Complexation by organic matter also detoxifies soluble aluminium in acid soils (Stevenson and Cole, 1999; Palm *et al.*, 2001).

Whereas the regulatory functions of soil organic matter on nutrient availability and acidity are most important in land-use systems with low addition rates of mineral fertilizers and lime, the stabilization of soil structure by organic matter is equally important in low as in high input agriculture. This is especially so in sandy soils where the stabilizing effect of clay on soil structure is limited by low clay contents (Pieri, 1989). In contrast, Ferralsols have an inherently favourable microaggregate structure which is little influenced by their organic matter content (Dudal and Deckers, 1993; Lehmann *et al.*, 2001a). The organic matter content

of a soil also influences its ability to store water, although this effect should not be overestimated (Gregory, 1988; de Ridder and van Keulen, 1990). For example, Diels *et al.* (2002) estimated that, in a soil with sandy loam texture, an increase in carbon content from 0.8 to 1.3% in the top 15 cm of the profile allowed an additional water storage of only 1 mm, compared with at least 50–70 mm of available water in the root zone of a 4-week-old maize crop.

Soil organic matter is also a substrate for soil biota, and it can help to control populations of plant pathogens in the soil by stimulating the activity of antagonistic microorganisms (Heitefuss, 1987). Recently, the potential role of soil organic matter as a sink for atmospheric carbon dioxide has received attention (van Noordwijk *et al.*, 1997).

Effects of land use on soil organic matter

Because of the importance of organic matter for soil fertility, the effects of land-use change, management practices and fallowing on the organic matter content of tropical soils have often been investigated. Numerous studies in the tropics have demonstrated loss of soil organic matter and organic nutrients following the conversion of forest or savanna ecosystems into either annual (Nye and Greenland, 1960; Pieri, 1989; de Ridder and van Keulen, 1990; du Preez and du Toit, 1995) or perennial cropping systems (Ollagnier *et al.*, 1978; Ahenkorah *et al.*, 1987; Ekanade, 1987). The reasons for this include a reduction in total organic inputs (litter, crop residues, manure) and increased mineralization rates of soil organic matter caused by tillage, increased soil temperatures due to exposure of the soil surface, and increased wetting-and-drying cycles. Organic matter may also be lost by soil erosion (Pieri, 1989; Palm *et al.*, 2001). Fallowing leads eventually to increases in soil organic matter content, reversing the effects of land clearing and cropping, although decreases may continue to be observed during the first 1 or 2 years of a new fallow (Szott *et al.*, 1999). In situations where soil organic matter content is either increasing or decreasing, new equilibria are reached more rapidly in sandy than in clay soils (Feller *et al.*, 1991b; du Preez and du Toit, 1995). Like fallows, trees in crop fields can contribute to the maintenance and improvement of soil organic matter levels through increased inputs of litter and roots, reduction of soil temperatures through shading, and soil protection from erosion. Favourable effects of agroforestry on the soil organic matter balance have been demonstrated in many studies (Young, 1997; Rao *et al.*, 1998).

Several management factors influence the rate of organic matter loss under cultivation in a manner that is often complex and difficult to predict. Additions of organic materials such as root biomass, manure and compost

have a favourable effect on soil organic matter (de Ridder and van Keulen, 1990). In contrast, the incorporation of cereal straw has been shown to stimulate microbial activity and cause carbon losses in sandy savanna soils in West Africa (Pieri, 1989). Measures that increase the amount of plant biomass above and below ground and its rate of accumulation tend to improve the organic matter balance by increasing carbon inputs to the soil and may also reduce erosion of otherwise incompletely covered soils, especially during the early part of the cropping season (Pieri, 1989). Most of the available data from trials in the West African moist savanna zone indicate that organic carbon contents of plots with fertilizer application are comparable to or slightly higher than those of unfertilized plots (Vanlauwe *et al.*, 2001). Sanchez (1976) also reports that long-term application of inorganic fertilizer delays the decrease in soil organic matter content under cropping by providing more crop residues, including roots. However, in contrast to the generally favourable effect of an equilibrated fertilization on soil organic matter, large applications of nitrogen fertilizer such as those recommended under intensive production of cereals have been shown to increase soil organic matter loss in the West African savanna (Pieri, 1989). For maintaining adequate levels of organic matter in the soil, sufficient amounts of organic inputs are needed, in the form of either crop residues, tree litter, compost, manure or a combination of these, depending on the land-use system. Long-term experiments on a range of soils in both West Africa and India show declining yields of a range of crops when only inorganic fertilizer is used for prolonged periods, suggesting that organic matter generally declines to below a critical level under continuous cropping unless there are organic additions (Greenland, 1994).

The effect of tillage on the soil organic matter balance is also variable, as tillage may increase crop yields and therefore carbon inputs into the soil, but also increases the microbial decomposition of soil organic matter and in some cases soil erosion (Pieri, 1989).

Functional pools of soil organic matter

As mentioned initially, soil organic matter is a mixture of living and dead components differing widely in their chemical composition and physical structure, which make them more or less resistant to microbial degradation. Some components are characterized by relatively rapid turnover and may contribute significantly to the mineralization and immobilization of nutrients. Examples are the microbial biomass and plant residues which have not yet undergone an intensive transformation in the soil (the light fraction or particulate organic matter fraction, see below). These fractions also respond rapidly to management measures. Humic substances, in contrast, are protected to some extent from microbial degradation by their

chemical structure, association with mineral particles and/or occlusion into aggregates. These substances make an important contribution to soil fertility through the stabilization of the soil structure and cation exchange processes, whereas their contribution to nutrient release is insufficiently known (Woomer *et al.*, 1994). Recently, increased attention has been given to finely divided charcoal as a component of the stable organic matter of many soils (Haumaier and Zech, 1995; Skjemstad *et al.*, 1996).

Considerable research effort is being invested in the identification and characterization of different pools of soil organic matter. The objective of these studies is to obtain an improved understanding of the transformations, stabilization and loss of soil organic matter, and of the relationships between the characteristics of certain organic matter fractions and their functions in soil, especially their nutrient supply properties. These attempts are associated with the development of computer simulation models, which divide soil organic matter into several pools with different turnover times to predict effects of site factors and management measures on carbon stocks, nutrient cycling and plant growth (see Box 4.1). For agroforestry, these efforts are relevant in so far as they may lead to prediction of the effects of certain agroforestry techniques, plant species and management measures on the soil carbon balance and associated processes, such as nutrient release and stabilization of soil structure.

Another application of soil organic matter fractionation is that, by quantifying those carbon fractions that respond rapidly to changes in vegetation cover or soil management, the effect of land-use change or experimental treatments on soil organic matter can often be detected more sensitively over the course of an experiment than when only total soil organic matter is measured.

Box 4.1. Modelling soil organic matter dynamics.

The amount of organic matter in a soil is a function of additions of fresh organic matter and decomposition rates, the latter being influenced by a series of intrinsic (e.g. residue quality, see Section 6.4) and soil-related controls (e.g. soil water content and temperature, composition and activity of the decomposer community and soil texture). Models describing soil organic matter dynamics usually contain a number of conceptually distinct pools, interconnected through a number of mass fluxes. A first set of pools is usually related to fresh organic matter and takes account of its quality, whereby higher quality is related to faster decomposition. Paustian *et al.* (1997) present a concise summary of mathematical approaches used to include residue quality in decomposition models. A second set of pools differentiates various soil organic matter fractions with varying turnover times. The microbial biomass, which is the soil organic matter pool with the highest turnover and through which most of the added organic carbon passes, is often one of these

Continued

Box 4.1. *Continued.*

fractions. Besides carbon, certain models such as CENTURY (Parton *et al.*, 1994) also simulate nitrogen and phosphorus dynamics.

The level of complexity of soil organic matter models increases with the number of pools and fluxes. Simple models usually contain a substantial amount of empirical information, obtained through calibration against available measurements for a certain environment. The validity of these parameters is obviously limited to that environment. More complex models contain a more important range of differential equations mechanistically linking various soil organic matter pools. A major advantage of simple models containing a minimal amount of pools and fluxes is that such models can be analytically solved and do not require simulation techniques. An example of a simple model which can be analytically solved is the ICBM model, containing only two pools, two decay constants, and parameters for litter input, humification and external influences (Andrén and Kätterer, 1997). An example of a model aiming at the mechanistic understanding of decomposition is the foodweb model of Hassink *et al.* (1994). As more complex models require more parameters to be calibrated, it is advisable to keep models as simple as possible if the modelling exercise is not the purpose of the study in itself.

Several reasons can be identified for embarking on soil organic matter simulation studies. First, as mentioned earlier, soil organic matter consists of a whole range of organic materials with varying resistance to microbial degradation and is continuously replenished by various sources of fresh organic matter. Summarizing available knowledge in the form of a simulation model can substantially help to understand how the different organic materials and soil organic matter pools interact and to develop research hypotheses. Secondly, after changes in land use, it can take several years before soil organic carbon reaches a new steady state. Although trials quantifying these changes in soil carbon are needed to calibrate the long-term modules of any soil organic matter model, it is clear that trials cannot be set up to deal with every possible land-use scenario. A well-calibrated model can be used to evaluate the impact of various land-use scenarios on the final soil organic carbon content and guide scientists and policy makers to develop guidelines favouring carbon build-up. Thirdly, models that quantitatively account for soil- and environment-related modifiers of the decomposition process allow extrapolation of carbon dynamics from the field scale to a wider region and assist in developing regional carbon profiles.

Several attempts have been made to link conceptual soil organic matter pools with experimentally determined soil organic matter fractions. The only soil organic matter fraction that has been rather successfully linked to experimental data is the soil microbial biomass. Other clear links between experimentally determined soil organic matter fractions and conceptual pools are rare, although the light particulate organic matter fraction (>100 μm, <1.4 g cm^{-3}, see Section 4.3) has been equated with the Added Organic Matter pool in the DAISY soil organic matter submodel (Mueller *et al.*, 1998). Balesdent (1996) successfully related physical separates of soil organic matter with the structural compartment of the Rothamsted carbon model, and Gaunt *et al.* (2000) proposed a physical soil fractionation scheme to obtain organic matter fractions suitable for modelling.

Most soil organic matter models have been developed based on information obtained under temperate conditions. Although it is often argued that processes are not fundamentally different between temperate and tropical regions (Jenkinson and Ayanaba, 1977), environmental conditions, soils and vegetation are so different that models developed under temperate conditions cannot be directly applied to tropical conditions without proper validation. Examples of models which have been successfully used to simulate carbon behaviour under tropical conditions are the CENTURY model (Parton *et al.*, 1994) and the Rothamsted carbon model (ROTHIC) (Jenkinson, 1990; Diels *et al.*, 2002).

Labile soil organic matter

Particular attention has been given to microbial biomass (see Section 4.5) and to the light fraction and particulate organic matter fraction, which are obtained from soil samples by either density separation or sieving or a combination of both (see Section 4.3). These carbon pools are characterized by relatively rapid turnover in the soil and have, therefore, been used to estimate an active, labile, nutrient-supplying fraction of soil organic matter (Palm *et al.*, 2001).

The microbial biomass represents only a few per cent of soil organic matter, but is essential for decomposition and nutrient release from organic materials and contributes to soil aggregation. Microbial biomass carbon can be used as an index of soil organic matter dynamics by relating it to total soil carbon and comparing with a local reference soil under natural vegetation (Gregorich *et al.*, 1994).

The light fraction and particulate organic matter consist mainly of plant residues, principally roots, with some residues of animals and microorganisms. The turnover time of these fractions ranges from a few days to a few years (Woomer *et al.*, 1994; Stevenson and Cole, 1999). Seeds and charcoal may also be present and may have to be removed as they do not belong to the labile organic matter (Barrios *et al.*, 1997). Although the light fraction contains most of the particulate organic matter, the two fractions are not identical in quantity and composition (Gregorich *et al.*, 1994). The light fraction may contain as much as 30–40% of the organic carbon of a soil (Gregorich and Ellert, 1993; Stevenson and Cole, 1999), but values of 10% or less have been reported for sandy savanna soils in Africa (Barrios *et al.*, 1997; Lehmann *et al.*, 1998b). An important part of the soil microbial and enzyme activity is associated with these fractions.

Light fraction and particulate organic matter respond rapidly to changes in soil management and are affected by litter inputs and decomposition conditions in the soil. The greatest amounts in the soil are measured immediately after the incorporation of plant residues. Decomposition in the soil progressively removes the most active, nutrient-

supplying components, so that later measurements include increasingly recalcitrant materials with low nutrient-supply properties (Palm *et al.*, 2001). These seasonal fluctuations in quantity and quality of the light or particulate organic matter fraction have to be taken into consideration when developing sampling plans and interpreting the results. In fact, a recent study reported increased particulate organic matter in a Ferralsol under a tree crop with slowly decomposing litter compared with trees that produced litter of higher quality (Lehmann *et al.*, 2001a), suggesting that this fraction contained mostly recalcitrant residues.

An alternative approach to the separation of a labile soil organic matter fraction which could help to circumvent some of the difficulties associated with the heterogeneous nature of physical fractions is selective oxidation with chemicals. Blair *et al.* (1995) used permanganate to separate the labile from the stable soil organic matter pool. Based on this two-pool fractionation, they proposed a carbon management index which is obtained by multiplying the carbon pool index (quotient of total carbon in the sample soil and in a reference soil) by the lability index (quotient of the labile and the stable fractions in the sample soil). The carbon management index was sensitive to different management regimes in a number of experiments (Blair *et al.*, 1997). Systematic comparisons of physical and chemical indices of labile soil organic matter pools for different pedoclimatic and management conditions are clearly needed. Combinations of both approaches are possible.

Formation of stable soil organic matter

As mentioned initially, the supply of nutrients is only one of several important functions of soil organic matter, others include the stabilization of soil structure, water-holding capacity and cation exchange properties. Therefore, the build-up of a labile, nutrient-supplying, soil organic matter pool, which is rapidly mineralized in the soil, is not the only objective of organic matter management in agroforestry, another objective of similar importance is the maintenance of a high content of stable organic matter. Therefore, a question of critical interest is how the build-up of stable carbon in the soil may be enhanced by, for example, the use of certain plant species in fallows or tree–crop associations, the treatment of biomass before it is applied to the soil (composting), and the method and timing of biomass application.

Unfortunately, the processes of the stabilization of organic matter in soil are still poorly understood, and it is not known which characteristics of an organic material determine whether it enters a labile fraction of soil organic matter and is rapidly mineralized, or is humified and ends up in a stable pool. Some studies have indicated that biomass with a higher C:N

ratio and lignin content results in more soil organic matter, although this has not always been confirmed (Palm *et al.*, 1997). In a decomposition study with biomass from three tree species in a sandy savanna soil in Togo, the biomass with the highest polyphenol:N ratio and the slowest decomposition (from *Senna siamea*) was the most efficient in increasing the carbon and nitrogen content of the silt and clay fractions of the soil (Lehmann *et al.*, 1998b). This concurs with the suggestion that materials rich in reactive polyphenols could increase soil organic matter formation (Palm *et al.*, 2001). In a study in Nigeria, slowly decomposing organic inputs with high C:N and lignin:N ratios were also shown to produce organic matter with a higher cation exchange capacity than other materials, suggesting that they could be useful for increasing the cation exchange capacity of highly weathered soils (Oorts *et al.*, 2000).

It is, however, likely that materials which are most efficient for soil organic matter formation such as those high in lignin and polyphenols, are not the same as those that release nutrients immediately and increase crop yields in the short term by decomposing quickly because they have a low (lignin + polyphenol):N ratio (see Section 5.2 and Chapter 6). Faced with this choice, farmers are likely to select the organic materials (or plant species) that offer the greater short-term benefits, rather than those that offer the greatest effect on the long-term soil carbon and nutrient balance. Biomass rich in both lignin and nitrogen could possibly combine soil organic matter formation with high nitrogen availability, but this remains to be confirmed by further research (Palm *et al.*, 2001). The outcome of such research would determine whether mixtures of tree species with contrasting properties, such as fast and slowly decomposing biomass, are desirable in agroforestry designs, to increase both labile, nutrient-supplying and stable pools of soil organic matter, or whether a single tree species can achieve both.

How much organic matter does a soil need?

For optimizing agroforestry techniques with respect to the maintenance of soil organic matter, it is important to know how much organic matter a given soil actually needs to remain fertile. The answer to this question certainly depends on the availability of technical substitutes for the different functions of organic matter in soil fertility, such as mineral fertilizer, irrigation, tillage and pesticides (van Noordwijk *et al.*, 1997). Pieri (1989) discusses first steps towards defining critical soil organic matter contents for the maintenance of soil biological functions in sandy savanna soils but concludes that reliable values cannot yet be given. Minimum levels of total carbon or certain organic matter fractions in the soil to maintain an adequate soil structure are discussed in Section 10.5.

4.2 Methods for Total Soil Organic Carbon

Total organic carbon in soil is measured with wet or dry oxidation methods (Anderson and Ingram, 1993; Tiessen and Moir, 1993). Dry oxidation with automated CN-analysers has the advantage that all carbon forms in the soil are included in the measurement and that nitrogen and eventually other elements can be measured simultaneously. Procedures for wet oxidation in acid dichromate solution are available either with or without external heating. In the latter case, the oxidation of organic carbon is incomplete, and a correction factor is applied, such as assuming that only 74% of the organic carbon has been oxidized. As the carbon recovery varies between soils in a usually unknown manner, this method should only be used for treatment comparisons with the same soil, but not across different soil types. Wet oxidation procedures largely exclude elemental carbon, such as charcoal, which can be an advantage in some situations, such as when studying the effect of management on organic matter in soils that contain large and/or variable amounts of charcoal. The importance of charcoal as a component of stable organic matter in soils is, however, increasingly recognized (see Section 4.1). Depending on the measurement method in wet oxidation and the combustion temperature in dry oxidation, carbonates are either included in the measurement (requiring acid pretreatment of the samples) or are not included (Tiessen and Moir, 1993).

If total organic matter stocks of different soils or land-use types are to be compared, it is important to measure not only the carbon concentration in the soil at different depths, but also the bulk density for each depth interval, so that total quantities of carbon per unit area can be calculated. Land-use changes sometimes lead to a different distribution of organic carbon in the soil and litter profile – for example, as a consequence of soil tillage or of differences between trees and herbaceous vegetation in root distribution and litter decomposition. To distinguish such distribution effects from changes in total soil organic matter stocks, the soil should be sampled to at least 1 m depth or to the underlying parent material or a hardened horizon (Hamburg, 2000). If the areas to be compared differ in bulk density, as occurs where land use has caused pronounced soil compaction, sampling to the same depth implies that subsoil with low carbon content is included in the profile with the more compact soil, and correction may be necessary (de Moraes *et al.*, 1996).

4.3 Physical Fractionation Methods

Physical fractions of soil organic matter can be obtained by separation into size classes (of primary particles or aggregates, see below), density classes

or with a combination of both techniques. Fractions of particular interest are the particulate organic matter (or macroorganic matter) and the light fraction because of their high activity and turnover rates (but see comments on these fractions in Section 4.1). Particulate organic matter can be defined as the fraction of 53–2000 μm (sand fraction) that is obtained by sieving, as in normal particle size analysis, but without pretreatments for the destruction of organic matter, carbonates and iron oxides (Gregorich and Ellert, 1993). The lower limit of the fraction differs between authors, e.g. 150 μm (Meijboom *et al.*, 1995) or 20 μm (Feller *et al.*, 1991a). The light fraction is normally obtained by density separation of soil in a liquid with a density of 1.4–2.0 g cm^{-3} (several organic and inorganic liquids are in use), in which organic particles that are not associated with soil minerals float, whereas those associated with mineral components sink (Gregorich and Ellert, 1993; Stevenson and Cole, 1999). Some workers have combined these two approaches of separating an active soil organic matter fraction and have used the coarse sand fraction instead of the whole soil for density separation (Meijboom *et al.*, 1995; Barrios *et al.*, 1997). Anderson and Ingram (1993) recommended separation of the light fraction from the bulk soil <2 mm by flotation in water (for simplicity, instead of denser fluids) and collection of the floating material in a 0.25 mm sieve. When this method is used, care needs to be taken to standardize the water flow rate; moreover, dispersion even of sandy soils is not complete and fractions obtained do not consist solely of materials larger than 0.25 mm (B. Vanlauwe, unpublished results). With all fractionation methods, but especially when combining different fractionation techniques, care should be taken not to produce a large number of fractions which then cannot be related to pools with distinct properties in the soil.

Authors have either dispersed the soil or not before physically separating soil organic matter fractions. Whether soil is dispersed and to what extent this is done depend on the purpose of the study. The major distinction is between whether the focus is on relationships between soil organic matter and primary mineral particles (sand, silt, clay), or on relationships between organic matter and soil structural properties, such as the physical protection of organic matter by macro- and micro-aggregates or effects of organic matter on the stabilization of the aggregate structure (Feller and Beare, 1997) (see also Section 10.5). For example, Six *et al.* (2000) used a wet sieving technique to separate aggregates and then dispersed the aggregates to quantify the free and the intra-aggregate particulate organic matter. Some soils are very difficult to disperse. Full dispersion of a Ferralsol to the level of primary particles, for example, may be both unpractical and undesirable.

Soil dispersion can be accomplished by shaking with glass beads, use of ultrasound or by using chemicals such as sodium hexametaphosphate or sodic resins (Feller *et al.*, 1991c). When using ultrasound, it is important

not to apply too much energy as this may cause redistribution of carbon towards the lower particle size classes. Feller and Beare (1997) recommend using ultrasound for only the 0–50 μm soil fraction and not for the bulk soil (0–2 mm) to avoid such redistribution effects. The fraction >50 μm can then be dispersed by shaking with glass beads or with chemical dispersants. Alternatively, a low amount of energy can be used for dispersing the bulk soil and then higher amounts for dispersion of the fractions (Amelung *et al.*, 1998).

Chemical dispersion techniques may invalidate further characterization of fractions. For example, dispersing the soil with hexametaphosphate precludes later determination of phosphorus, so sodic resins can be used as dispersants in such studies (Feller and Beare, 1997). When using chemicals, it is important to standardize the shaking procedure on the basis of preliminary tests. For example, for a number of sandy savanna soils, the most complete dispersion with minimal redistribution of carbon towards the smaller particle size classes was achieved by shaking the soil in a sodium hexametaphosphate–sodium carbonate solution on a reciprocal shaker for 16 h at 144 rpm with a shaking amplitude of 4.5 cm (Vanlauwe *et al.*, 1998b, 1999). To calibrate a certain dispersion procedure, organic matter with a size similar to particulate organic matter, or particulate organic matter extracted from another soil sample, can be added to the sample and its carbon content traced after dispersion. ^{13}C- or ^{14}C-labelled materials allow an accurate tracing of the added carbon. For an extensive review of physical fractionation approaches and results from tropical soils see Feller and Beare (1997).

4.4 Chemical Methods

The plant, faunal and microbial residues from which soil organic matter is derived consist of identifiable chemical substances such as carbohydrates, lignin, fats, waxes and proteins. These are decomposed and transformed in the soil through microbial and faunal action, and new substances are synthesized. The chemical composition and transformations of soil organic matter are studied with a number of specialized techniques, including chromatographic methods, nuclear magnetic resonance spectroscopy and analytical pyrolysis. These methods have been reviewed by Stevenson and Cole (1999) and Skjemstad *et al.* (1997), and recent results from tropical soils are given by Golchin *et al.* (1995), Skjemstad *et al.* (1997) and Zech *et al.* (1997). Results of such studies are potentially very relevant to agroforestry research, but their application is restricted to specialized laboratories.

Easier to apply are techniques that attempt to separate labile fractions of soil organic matter through selective oxidation without chemically

characterizing these fractions. As mentioned in Section 4.1, the separation of a labile organic matter pool through oxidation with a 333 mM solution of potassium permanganate ($KMnO_4$) has been proposed by Blair *et al.* (1995). Modifications of the method, using less concentrated $KMnO_4$ solutions, have been suggested by other workers. Bell *et al.* (1998) found that the carbon fraction oxidized with a 33 mM solution of $KMnO_4$ was more closely related to aggregate stability and (in combination with pH) effective cation exchange capacity of a range of Australian soils than that obtained with more concentrated solutions (for use of this fraction to predict rainfall infiltration and runoff see Section 10.5). Greater sensitivity of the fraction oxidized with a 33 mM than with a 330 mM $KMnO_4$ solution for detecting changes in the organic matter quality of cultivated soils in north-eastern Brazil was reported by Shang and Tiessen (1997). It should be noted that chemical fractionation of soil organic matter with permanganate destroys the respective fraction, whereas physical fractionation allows the further characterization of the isolated fractions.

Soil organic matter has traditionally been separated according to its solubility in acid and alkali into humic acids, fulvic acids and insoluble humin (Anderson and Schoenau, 1993; Stevenson and Cole, 1999). However, these fractions are not closely related to soil organic matter functions and are not generally used in process-oriented research.

4.5 Biological Methods

For the measurement of soil microbial biomass, the fumigation–extraction method, which allows the simultaneous measurement of microbial nitrogen, phosphorus and sulphur, and various other methods are discussed by Voroney *et al.* (1993), Stevenson and Cole (1999) and Carter *et al.* (1999). Soil microbial biomass methods are prone to error when applying them after recent applications of fresh organic matter, which is a problem in agroforestry research. The magnitude of overestimation of microbial biomass after application of organic matter depends on the chemical characteristic that is measured to obtain microbial biomass. Vanlauwe *et al.* (1994) found that, when estimating microbial biomass from the concentration of soluble carbon in the soil before and after the fumigation, microbial biomass was substantially overestimated for at least nine days after residue application. When microbial biomass was estimated from ninhydrin-reactive nitrogen, overestimates were smaller and did not last as long. For nutrients in microbial biomass see the respective sections in Chapter 5.

Soil respiration measurements provide an index of soil organic matter quality if the amount of carbon dioxide released is related to the total carbon present in the sample (Gregorich *et al.*, 1994). Soil respiration can be measured in the field or under standardized conditions in the

laboratory. The latter approach was used for comparing the organic matter quality in soils from different leguminous tree fallows (Schroth *et al.*, 1995b). In this study, the most sensitive indicator of species differences was the mineralization flush on the first day after rewetting the samples, which tended to increase with the litterfall of the tree species. Methods of separating root and microbial components of soil respiration are discussed by Hanson *et al.* (2000).

The use of soil enzymes for assessing soil organic matter quality is discussed by Gregorich *et al.* (1994) and Stevenson and Cole (1999). For more detailed discussions of soil microbiological methods see Chapters 13 and 15.

Box 4.2. Identifying soil organic matter sources with carbon isotopes.

The amelioration of soil organic matter by trees is an important goal in agroforestry. Carbon isotope techniques can in some cases identify the source of soil organic matter by tracing the origin of its carbon. Plants that are cropped together or in sequence often possess different metabolic pathways for the assimilation of carbon dioxide from the atmosphere: most trees, legumes and root crops use the so-called C_3 pathway, whereas savanna grasses, maize and sorghum use the C_4 pathway. The metabolic pathway used by a plant affects the ratio of the two carbon isotopes, ^{12}C and ^{13}C, in its biomass, because C_3 plants discriminate more against the heavy ^{13}C isotope than C_4 plants and their biomass has therefore a lower ^{13}C:^{12}C ratio. The carbon isotope ratios of the soil organic matter that is derived from this biomass vary accordingly.

The stable isotopes ^{13}C and ^{12}C can be measured by dry combustion of a plant or soil sample and subsequent mass spectrometry. The ^{13}C:^{12}C ratio of a sample is conventionally given in relation to a standard, usually the Pee Dee Belemnite (PDB), and expressed in parts per thousand (‰) as the $\delta^{13}C$ value:

$$\delta^{13}C = \left(\frac{\frac{^{13}C}{^{12}C_{(sample)}}}{\frac{^{13}C}{^{12}C_{(sample)}}} - 1 \right) \times 10^3 \quad [‰] \tag{4.1}$$

Plants with the C_3 pathway have $\delta^{13}C$ values in the range of –35 to –20‰, whereas plants that use the C_4 pathway have $\delta^{13}C$ values between –19 and –9‰ (Boutton, 1991). Because of the isotopic difference between C_3 and C_4 plants, carbon isotopes can be used for measuring the effect of vegetation change on soil organic matter, if either C_3 plants were introduced into an area that was previously dominated by C_4 species, or C_4 species were introduced in a previously C_3

dominated vegetation. If a C_3 species (e.g. a legume tree) is cultivated in a native C_4 vegetation (e.g. grassland), the contribution of the introduced tree to soil organic matter can be calculated as:

$$f_{(C3)} = (\delta^{13}C_{(C3)} - \delta^{13}C_{(C4)})/(\delta^{13}C_{(C3^*)} - \delta^{13}C_{(C4^*)}) \quad (4.2)$$

where $f_{(C3)}$ is the proportion of soil organic matter derived from the C_3 tree species; $\delta^{13}C_{(C4)}$ is the $\delta^{13}C$ value of the soil under the original C_4 vegetation (control grassland plot); $\delta^{13}C_{(C3)}$ is the $\delta^{13}C$ value of the soil under the introduced C_3 vegetation; $\delta^{13}C_{(C4^*)}$ and $\delta^{13}C_{(C3^*)}$ are the $\delta^{13}C$ values of the soil organic matter derived from the two plant species, respectively. The $\delta^{13}C$ value for the soil organic matter derived from the original vegetation ($\delta^{13}C_{(C4^*)}$) can be taken from the control plots covered by grassland. This is rarely possible for the introduced plant ($\delta^{13}C_{(C3^*)}$), because no fields exist at the same site which have been cropped with the tree species for the same time as the original grass vegetation. This value is usually substituted by the $\delta^{13}C$ value of the plant material, either the aerial parts (for topsoil) or the roots (for subsoil) (Schweizer *et al.*, 1999). This is a valid approach, as the discrimination between the two carbon isotopes during mineralization and humification of plant material is believed to be negligible in the topsoil, although it may be of significance in the subsoil (Boutton *et al.*, 1998).

The $\delta^{13}C$ value can be affected by environmental factors such as water stress, but these effects are much smaller than the differences between C_3 and C_4 vegetation (Boutton, 1996). Therefore, the isotope composition of the soil organic matter in the topsoil reflects that of the living biomass or litter from which it is derived. Care has to be taken to remove carbonate, if present, from soil prior to analysis without changing the $\delta^{13}C$ values of the soil organic matter (Midwood and Boutton, 1998).

Carbon isotope methods have been used to assess the effect of land-use changes on soil organic matter quality (Feigl *et al.*, 1995), determine soil organic matter turnover rates (Bernoux *et al.*, 1998), reconstruct vegetation history at the landscape scale (Boutton *et al.*, 1998), measure the contribution of root vs. shoot tissue to soil organic matter formation (Balesdent and Balabane, 1996) and separate soil respiration into contributions from soil organic matter and added green manure (Nyberg *et al.*, 2000). In an area in western Kenya that had previously been occupied by C_4 crops or pasture grasses, a comparison of $\delta^{13}C$ values of topsoil under the canopy of different tree species (C_3) and outside the tree canopy detected significant effects of the trees on soil carbon as early as 5 years after tree planting (Nyberg and Högberg, 1995). Similarly, the contribution of scattered trees to soil organic matter in the Sahel savanna of Burkina Faso was quantified by comparing carbon isotope ratios in soil collected under the trees and in the open (Jonsson *et al.*, 1999). Other agroforestry situations where the technique might be useful include rotations between C_4 crops and C_3 trees in fallow systems, and associations of C_4 crops and C_3 trees. A method for separating the effect of C_4 grasses and C_3 legumes on soil organic matter in a mixed grass–legume pasture after rainforest has been developed by Cadisch and Giller (1996).

Chapter 5
Soil Nutrient Availability and Acidity

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5.1 Synopsis

In most tropical soils, the growth and production of crops is limited by the availability of one or several nutrients, such as nitrogen in sandy savanna soils and phosphorus in most acid soils (Ewel, 1986; von Uexküll, 1986). Especially in humid climates, nutrient deficiency is often associated with soil acidity and aluminium toxicity, which may reduce the root development of plants and further aggravate the problems of insufficient nutrient supply from the soil. In dry climates, nutrient deficiency can limit the ability of crop and fodder plants to make use of the relatively short periods when there is sufficient soil moisture for plant growth (Breman and Kessler, 1997).

Under favourable economic conditions, including the availability of credit, access to agrochemicals at reasonable prices, and a lucrative market for agricultural products, soil nutrient deficiency and acidity can be corrected through fertilizing and liming, and productive agriculture can then be possible even on previously very infertile soils. An example of this is the large-scale transformation of the acidic, nutrient-poor Oxisols of the Brazilian *cerrado* savannas into productive cropping systems through large inputs, especially of phosphorus fertilizer and lime (Sanchez, 1997).

Many smallholder farmers in the tropics, however, can only afford to use small quantities of mineral fertilizers or sometimes none at all, and sufficient amounts of organic fertilizers, such as farmyard manure, are rarely available. In any case, with the exception of nitrogen fixed by leguminous fodder plants, the nutrients contained in manure would

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mainly constitute a redistribution of nutrients from other parts of the farm or landscape and not a true nutrient addition. Insufficient fertilizer applications to compensate for the nutrient exports from the farm in harvested products as well as nutrient losses from soil erosion, leaching and fire result in the widespread occurrence of negative nutrient balances in tropical farming systems (see Chapter 1).

Nutrient efficiency

As a consequence of low initial soil fertility and insufficient access to mineral and organic fertilizers to correct nutrient deficiencies and compensate for nutrient exports and losses, smallholder agriculture is widely practised under nutrient-limited conditions in the tropics. To be a viable land-use form under such conditions, agroforestry systems have to make efficient use of the nutrients available in the soil and of nutrient additions in mineral and organic fertilizers so that they are productive even if the nutrient supply is much lower than requirements for optimum growth. This should not be misunderstood to suggest that agroforestry systems could remain productive in the long term (i.e. be sustainable) without external nutrient additions to compensate for nutrient exports and losses, except – for a certain time – on very fertile soils or under conditions of very low yields. This is especially so for nutrients such as phosphorus that are only added to terrestrial ecosystems in very small quantities through atmospheric deposition or weathering of primary minerals (see Section 5.3).

As a comparative measure of the ability of plant species or varieties to grow and produce yields on nutrient-limited soils, agronomists have developed the concept of *nutrient efficiency* (Marschner, 1995). A crop species or variety A is said to be more nutrient efficient than a species or variety B if A reaches a satisfactory level of productivity at a lower nutrient supply than B, even if both have the same productivity when nutrients are not limiting. Being more nutrient efficient, A can be grown on a less-fertile soil and with less fertilizer input than B. Differences in nutrient efficiency between plant species can be large: Marschner (1995) mentions three pasture species which needed 302, 87 and 26 mg P kg^{-1} of soil to produce 90% of a common maximum biomass yield (this does not take into consideration eventual differences in fodder quality). The concept can also be applied to specific yield components, such as grain, instead of total biomass.

Nutrient efficiency has two components: the *efficiency of nutrient acquisition* by the roots as measured by total nutrient uptake per plant, and the efficiency of nutrient utilization within the plant, which is called *nutrient-use efficiency* and is defined as the dry matter produced per unit nutrient in the dry matter. A large root system, efficient association with

mycorrhizas and abundant nodulation in nitrogen-fixing plants are examples of characteristics that can lead to efficient nutrient acquisition. Efficient nutrient translocation within the plant and low nutrient requirements on the cellular level are factors that increase the nutrient-use efficiency. Alone or in combination, such characteristics tend to increase the productivity of plants when grown in soil with low nutrient availability (Marschner, 1995).

Farmers with infertile soils will be interested in growing crop species with a high nutrient-use efficiency. For example, C_4 grasses make more efficient use of nitrogen and, therefore, have lower nitrogen concentrations in their tissue than C_3 grasses, and grasses in general make more efficient use of calcium and boron than dicotyledons. There are also differences between varieties (Marschner, 1995). In contrast to herbaceous crops, high nutrient-use efficiency is not generally a suitable selection criterion for agroforestry trees because, in addition to their production functions, the trees are often expected to increase nutrient cycling within the system, and a smaller amount of nutrients will be cycled if the trees have low nutrient concentrations. This function of agroforestry trees explains in part why the use of legume trees with their typically high nutrient concentrations is so common in tropical agroforestry. Suitable agroforestry tree ideotypes for situations where enhancing soil fertility is an objective would be both reasonably fast-growing and possess high tissue nutrient concentrations, which implies efficient mechanisms of nutrient acquisition rather than a particularly high internal nutrient-use efficiency.

Effects of agroforestry practices on nutrient cycles

The effects of agroforestry practices on nutrient cycles are the topic of several chapters of this book, and only a short overview is given here. A terminology for the analysis of nutrient cycles in agroforestry and other land-use systems is proposed in Box 5.1.

Agroforestry practices can increase the total quantity of nutrients in the soil–plant system by increasing the nutrient transfers into the system and by reducing nutrient losses from the system. Increased nutrient inputs may originate either from the atmosphere or from soil compartments which are outside the reach of crop plants. The most important example for increased nutrient inputs from the atmosphere into agroforestry systems is biological nitrogen fixation by legume or other nitrogen-fixing trees (see Chapter 13). Trees can also increase atmospheric nutrient deposition but the quantities involved are usually small (see Chapter 9). If deep-rooting trees are associated or grown in rotation with shallow-rooting crops at a site where nutrients are available in deeper soil horizons, nutrients which were previously below the crop rooting zone may be made available to the

crops via tree litter or prunings. The same applies if tree roots reach laterally more distant soil and take up nutrients which are not accessible to the crop roots, e.g. in a stream bank or in a neighbouring plot or farm. Processes of nutrient capture that involve an increase in the effective rooting volume of a system through tree integration are discussed in Chapter 8.

Agroforestry techniques can also help farmers avoid unproductive nutrient losses from a land-use system, i.e. losses of nutrients that are not exported in harvested products. Contour hedgerows and a continuous soil cover reduce soil erosion and surface runoff on sloping land (see Chapter 17), and the permanent or intermittent presence of deep tree roots can help to reduce nutrient leaching, especially during times of the year when no crops are present (see Chapter 7). Permanent associations of crops with trees also oblige the land user to protect the field from fire and associated nutrient losses (see Chapter 9), although tree planting in fallow rotations does not necessarily have this effect (MacDicken, 1991).

Trees could also increase the total amount of nutrients cycling in a system by accessing nutrient pools that are not accessible to crops, for example through more efficient mycorrhizal associations (see Chapter 15) or solubilization of recalcitrant phosphorus forms in the rhizosphere (see Chapter 15). However, such effects are not yet well researched.

Box 5.1. A terminology for studying nutrient cycles of agroforestry systems.

For the conceptual analysis, experimental study and modelling of nutrient cycles in agroforestry systems, it is useful to subdivide the total nutrient quantity within a system into pools and compartments as illustrated in Fig. 5.1.

Nutrient pools

A nutrient pool is defined here as a fraction of the total amount of a given nutrient in the plant–soil system whose chemical, physical or biological properties differentiate it from other fractions of the same nutrient with respect to reactivity, mobility and availability to plants, animals or microorganisms. In an agricultural or agroforestry system, the nutrients in the biomass, necromass and soil constitute separate pools, and each of these can be further subdivided as needed for the purpose of a study. In particular, the different forms of a nutrient in the soil are often subdivided into various pools, commonly organic vs. inorganic and labile vs. stable (Fig. 5.1). The differentiation between pools of the same nutrient is sometimes clear-cut and sometimes rather arbitrary and dependent, for example, on the extraction method used.

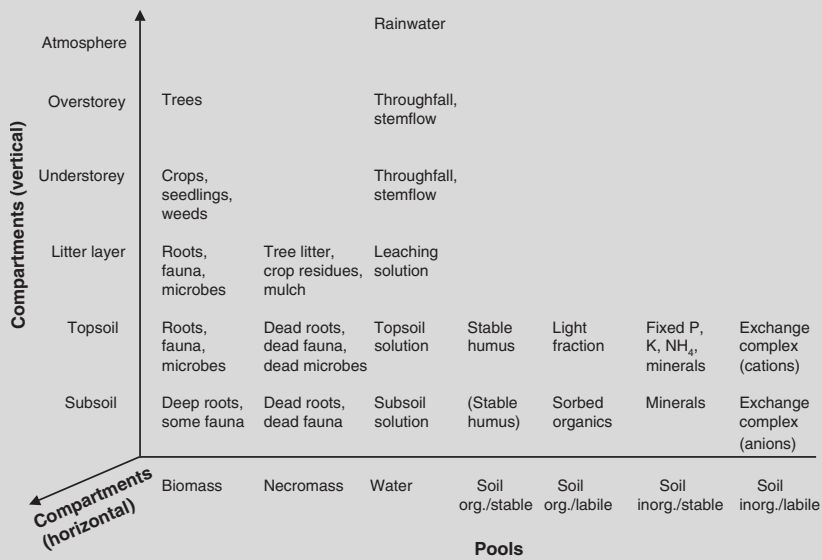


Fig. 5.1. Schema of the subdivision of total nutrient stocks in agroforestry systems into pools and vertical and horizonal compartments according to their physicochemical form and location. org., organic; inorg., inorganic.

Compartments

In contrast to pools, which differentiate between physicochemical or biological forms of a nutrient, nutrient compartments subdivide the total quantity of nutrients within a system according to their location (Fig. 5.1). For example, the subdivision of the soil profile into topsoil and subsoil is useful in an agroforestry study if the topsoil is defined as the rooting zone of shallow-rooted crops, and the subsoil is defined as the soil not accessible to these crops but still within the reach of trees. Nutrients in the shoots and roots are assigned different compartments, because they differ in their susceptibility to loss from the system by harvest or fire and their ease of management through, for example, pruning. A further subdivision of the system into horizontal compartments is useful in simultaneous agroforestry systems. Specifically, nutrients in the soil directly under trees and crops may be distinguished from each other and from nutrients at a distance too far to be reached by crop roots but accessible to tree roots that have greater lateral extension. The most important criterion for the distinction of both pools and compartments is that they are useful for the conceptualization, measurement and modelling of the nutrient cycles in the plant–soil system as influenced by land-use practices.

Continued

Box 5.1. *Continued.*

Nutrient transfers

A nutrient transfer is a physical movement of a nutrient into or out of a system (typically a plot or a farm) or between different compartments within a system. Examples of nutrient transfers across the plot boundary include imports of nutrients with rainfall or dust and nutrient losses with fire. Leaching of a nutrient from the topsoil into the subsoil or nutrient returns from the vegetation to the soil via throughfall, stemflow and litterfall are examples of nutrient transfers between different compartments within a system. Nutrient transfers are often called fluxes but this term is also commonly used to refer to exchanges among pools (see Box 4.1).

Nutrient transformations

Nutrient transformations are exchanges of nutrients between pools. Unlike nutrient transfers, they do not involve a major physical movement of the nutrient, but rather one or several biochemical or physicochemical reactions that change the reactivity, mobility and/or availability of the nutrient. Examples of nutrient transformations include the mineralization or immobilization of nitrogen, phosphorus and sulphur in litter and soil, or exchanges between physicochemical forms of phosphorus and potassium which differ in their reactivity and availability to plants.

Nutrient transformations and transfers often affect each other. For example, nitrification in the topsoil (a transformation) strongly increases nitrogen leaching into the subsoil compartment (a transfer).

Nutrient efficiency and tree–crop interactions

Through the mechanisms mentioned above, the total amount of nutrients that are taken up from soil and air by a tree–crop association may be larger than that available to a crop alone. If the integration of deep-rooting, nitrogen-fixing, highly mycorrhizal trees into a field with annual or perennial crops increases the total nutrient uptake and biomass production of a land-use system on a nutrient-poor soil, then the practice has been successful in terms of improved nutrient efficiency. However, it can still be an economic failure. Although the trees may increase the acquisition of certain soil resources and the biomass production of the system, they may also compete with the crops for the same and other resources, and the overall economic output of the association may be less than that of the crop alone.

The partitioning of soil resources between trees and crops, and therefore their respective productivity in an association, depends on the interactions between the associated species. These interactions can be

complex, involving a large number of processes and their spatiotemporal patterns, and are rarely fully understood for a given association. For their conceptualization and analysis, it is useful to distinguish between three principal types of interactions:

- with competition, uptake of a nutrient by species A reduces its availability for species B;
- with complementarity, uptake of a nutrient by species A does not affect its availability for species B, so that nutrient uptake by species A and B is additive;
- with facilitation, species A increases the availability of the nutrient for species B.

Details and examples for these types of interactions are given in Box 5.2.

In simultaneous agroforestry systems, these types of interactions rarely or never occur in isolation in the field. For example, a deep-rooting tree may utilize leached nitrate in the subsoil that is not accessible to an associated annual crop, and the interaction between the two species with respect to this nitrate would then be complementary and beneficial for the system as a whole. However, the tree would also acquire some of its nitrogen as well as other nutrients and water from the topsoil, and here the interaction with the crop could be competitive. Competition in the topsoil could even be an important incentive for the tree to form deep roots and therefore a precondition for complementarity between the two species in the subsoil. Similarly, the integration of nitrogen-fixing trees into a cropping system on a nitrogen-poor soil can increase the total nitrogen acquisition by the system, and the trees could have a facilitative effect on the crops through the release of nitrogen from tree prunings, but could at the same time compete with the crops for other nutrients, water, light or simply space.

Whether an association is successful or not depends on the balance between competitive, complementary and facilitative interactions on the one hand, and on the relative value of tree and crop products on the other hand (van Noordwijk and Purnomosidhi, 1995). The higher the value of the trees, the more competition with the crops will be accepted, and the lower their value, the more the resource use by trees and crops needs to be complementary or facilitative. In many agroforestry associations, the (short-term) benefit from the crops will be far higher than that from the trees, and little competition will therefore be tolerated. Besides requiring time and sometimes monetary inputs, in most cases tree planting reduces the space available for the crops. Therefore, planting trees with no direct economic value (e.g. certain legume trees) can only be economically advantageous if they exert a net facilitative effect on the crops. Furthermore, this effect would have to become perceptible over a relatively

short time period to provide an incentive for tree planting (see Chapter 2). Failure to take these considerations into account may help to explain the low adoption of some agroforestry techniques, such as alley cropping.

Box 5.2. Types of interactions between plants.

There are many ways in which plants growing on the same piece of land can interact. Of particular relevance for understanding and managing nutrient and water cycles in agroforestry are three types of interactions: competition, complementarity and facilitation. In agroforestry systems, these types of interactions generally occur simultaneously and may influence each other (see text).

Competition

A major problem in agroforestry associations is competition between trees and crops for light, water and nutrients. Competition for a nutrient occurs when two species attempt to meet their demand for this nutrient from the same pool and compartment in the soil (see Box 5.1), and when their combined demand exceeds the supply during a certain time interval. Some competition is probably inevitable where overall resource use is efficient (for example, to reduce nutrient leaching, an intensive, interlocked root system is needed), but excessive competition leads to the suppression of plants and low yields. It is, therefore, a general objective in agroforestry to avoid strongly competitive relationships between species and to create complementary or even facilitative relationships.

There are several conceptually important distinctions within the term competition. *Intraspecific competition* occurs between individuals of the same species, and *interspecific competition* between individuals of different species. Everything else being equal, the former is commonly assumed to be more intense than the latter because individuals of the same species have exactly the same resource requirements and uptake patterns (i.e. they occupy the same niche). *Exploitation competition* occurs when plants compete directly for the same nutrients or water in the soil, i.e. when the nutrient or water depletion zones around their roots overlap. *Interference competition*, on the other hand, occurs when one individual indirectly impedes the access of the other individual to the limiting resource, for example by producing allelopathic substances which reduce root growth of competing species. Finally, *size-symmetric competition* describes the situation when two competitors obtain the contested resource in proportion to their size, whereas, with *size-asymmetric competition*, the larger plant obtains a disproportionate share of the resource. Competition for light is usually strongly asymmetric (a plant needs to be only a little taller than its neighbour to obtain the lion's share of the incoming radiation), whereas competition for nutrients seems to be more symmetric. Little is known about the symmetry of competition for water (Schwinning and Weiner, 1998).

Complementarity

Complementarity between two interacting species means that the use of a resource by one species is not at the expense of the use of this resource by the other species. With respect to soil nutrients this occurs when two species use different pools or compartments of a nutrient, or when they use the same pool or compartment at different times. This is a desirable situation, because the two species in combination would then tend to use this nutrient more effectively than either species alone. Several examples for complementary nutrient use in agroforestry systems have been mentioned in the text: capture of nutrients from the subsoil or laterally distant soil by trees, differential access to atmospheric nitrogen in associations of nitrogen-fixing and non-nitrogen-fixing species, and differences in the access to recalcitrant nutrient pools in the soil. Complementarity through the temporal separation of nutrient uptake would be expected when associated species differ in their phenology and more markedly in rotational systems when plants are deliberately grown at different times.

Facilitation

Facilitation means that one species actually improves the growth conditions of the other species. There is a substantial literature on facilitative interactions in natural plant communities (Callaway, 1995; Callaway and Walker, 1997). In fallow rotations, facilitation of crop growth by the fallow trees is the expected consequence of the accumulation of nutrients, including fixed nitrogen, in the biomass during the fallow phase and their release following the reconversion of the fallow into a crop field. Facilitation can also be achieved through the application of nutrient-rich tree prunings to crops in a tree–crop association, provided that the nutrients released from the prunings are predominantly taken up by the crops and not reabsorbed by the trees themselves.

Optimization of nutrient cycles in agroforestry

The tools that are available to a farmer for optimizing nutrient cycles are the selection of plant (and animal) species, their spatial and temporal arrangement (system design), and their management.

The selection of crop species, be they herbaceous or perennial, is generally dominated by market and household constraints, but considerable flexibility often exists with respect to the selection of service trees that are grown in association with them. Tree species differ with respect to their growth rates (and thus nutrient uptake), root distribution (and thus access to subsoil nutrients), litter production (and thus nutrient return to the soil), litter quality (and thus decomposition dynamics), ability

to fix nitrogen and many other characteristics that affect nutrient cycles. As mentioned before, high nutrient-use efficiency may be an economically important selection criterion for crop species and varieties in agroforestry systems, but may not be a good guide for selecting tree species, because the production of nutrient-rich, easily decomposable litter is a desirable property even for trees that serve predominantly production purposes. However, trees with high nutrient-use efficiency (i.e. low tissue nutrient contents) and high lignin and polyphenol contents could be useful for soil organic matter build-up (see Chapter 4).

Nutrient cycles are greatly affected by the way trees and crops are arranged in space and time (system design). Trees can be planted or allowed to regenerate in association with crops, as in shaded coffee and cocoa plantations, parkland systems, contour hedgerows and boundary plantings, and in each case the relative access of trees and crops to nutrients in the soil can to some extent be influenced through tree spacing and planting design. Trees can also be grown in rotation with crops, as in fallow systems. Trees may enhance nutrient cycling in both simultaneous and rotational systems. However, in the former case, they access at least partly the same nutrient pools and compartments in the soil as the crops (see Box 5.1) and may cause competition, although their net effect may still be beneficial because of microclimatic protection of shade-demanding crops, timber production, soil conservation or biological nitrogen fixation. In fallow systems, in contrast, trees and crops take up nutrients from the same soil at different times. Improved fallows are, therefore, seen as a means of minimizing competition while maximizing facilitative effects of trees on subsequent crops. Some competition between fallows and neighbouring crop plots may, however, occur through lateral tree roots in the small-scale patchwork of fallows and crop fields that is typical for tropical smallholder agriculture (van Noordwijk, 1999). Fallow systems permit the use of fast-growing tree species that would be too competitive for use in simultaneous tree–crop associations (Schroth *et al.*, 1996), and these can be planted at narrow spacing to achieve rapid biomass accumulation, soil regeneration and weed suppression without the need to be held in check by periodic pruning.

Agroforestry systems can be managed so as to reduce competitive interactions and increase complementary and facilitative interactions between associated tree and crop species (Lehmann, 2002). However, care is needed in deciding what measures to apply and how to apply them. Periodic shoot pruning of trees and transfer of the biomass to crops, as in tree crop plantations shaded by legume trees or in hedgerow intercropping, reduces nutrient sequestration in the tree biomass, improves the quality of the tree biomass (more and younger leaves, less old wood) and makes nutrients available to the crops, beside reducing the competition of the trees for water and light. Frequent pruning of trees

during the rainy season can cause an 'anti-cyclic' root development of the trees by shifting the period of maximum tree root growth into the dry season, thereby increasing the potential for complementarity with annual crops in the use of soil resources (Schroth and Zech, 1995b). However, the root systems of frequently pruned trees have also been found to become shallower (see Section 8.1), and nitrogen fixation of legume trees may be impeded by frequent, intensive shoot pruning (see Section 13.1). Similarly, soil tillage at the beginning of the cropping season destroys tree roots in the topsoil and gives the developing crop a temporary advantage in the competition for soil resources, but may also provoke increased mineralization of soil organic matter and nutrient release at a time when the crops are too small to absorb these nutrients.

Matching agroforestry practices to sites

As mentioned in Chapter 1, it is important to keep in mind that tropical sites and soils differ widely in their fertility, including the availability of different nutrients, and that agroforestry practices differ in their potential to adapt to, and eventually to mitigate, certain fertility problems. This means that not only crop and tree species, but also agroforestry techniques have to be matched to sites according to their respective potentials and limitations. For example, on sandy savanna soils, low nitrogen availability is often a limiting factor for crop production, indicating that agroforestry with nitrogen-fixing trees can contribute to improved soil fertility and higher yields (Barrios *et al.*, 1997). For certain forest soils, in contrast, nitrogen is less limiting, and the introduction of nitrogen-fixing species would not be given high priority (Schroth *et al.*, 1999a). On shallow soils, which are common in West Africa, root competition between trees and associated crops can be particularly intensive. Under these circumstances, simultaneous agroforestry systems may only be viable if uncompetitive tree species are used (Schroth and Lehmann, 1995) and improved fallows may be preferable. At sites where nutrient accumulation in the subsoil has been detected, agroforestry techniques can be specifically designed for the recycling of these nutrients, for example, through high-density plantings of fast-growing tree species as improved fallows (Jama *et al.*, 1998).

It should also be mentioned that in the tropics, agroforestry techniques are used not only under nutrient-limited conditions, but also in systems with relatively high fertilizer inputs, such as certain shaded coffee plantations (Beer *et al.*, 1998). Under such conditions, trees can still have beneficial effects on nutrient cycles, especially through soil protection and the reduction of nutrient leaching (Schroth *et al.*, 2001b).

5.2 Methods for Soil Nitrogen (*E. Barrios, G. Schroth*)

The prominent role that nitrogen occupies among the nutrient elements is because of the relatively large amounts of it that are required by plants and soil microorganisms in comparison with other nutrients. Low soil nitrogen availability limits crop growth on many tropical soils, especially those with low organic matter contents such as sandy soils and soils of semiarid environments. On such soils, the availability of nitrogen to crops can be effectively increased through the use of nitrogen-fixing trees in tree–crop associations or improved fallows. Nitrogen deficiency in plants is readily recognized by yellow coloration of the older leaves and by slow and stunted growth (Stevenson and Cole, 1999).

More than 95% of the nitrogen in topsoil is usually present in organic forms. Through microbial mineralization it is transformed into ammonium (NH_4^+) and then further oxidized by nitrifying soil microbes to nitrite (NO_2^-) and nitrate (NO_3^-), a process called nitrification. Recent results suggest that the opposite process, the dissimilatory reduction of nitrate to ammonium by microbes, which is usually assumed to occur only under flooded conditions, may be a relevant process also in upland tropical forest soils (Silver *et al.*, 2001).

Plants absorb most of their nitrogen from the soil as ammonium or nitrate, with nitrite usually being present only in minor quantities in well-aerated soils. However, both trees and herbaceous plant species have the ability to take up certain organic nitrogen forms, especially when they are associated with mycorrhizal fungi (Turnbull *et al.*, 1995; Näsholm *et al.*, 2000).

Burning of vegetation as in shifting cultivation can lead to large losses of biomass nitrogen (see Section 9.1). As nitrate, nitrogen is also easily lost from agricultural soils through leaching, especially following the mineralization peak that typically occurs at the beginning of the rainy season in seasonally dry climates (see Section 7.1). Recycling of leached nitrate from the subsoil can be an important function of trees in agroforestry systems (see Section 8.1). Nitrogen losses also occur through denitrification (Granli and Bockman, 1994; Barton *et al.*, 1999) and volatilization of ammonia, for example from decomposing biomass or surface-applied fertilizer, especially urea.

The amount of mineral nitrogen that is available to a crop during its life cycle can be calculated as the mineral nitrogen present in the soil at the beginning of the cropping season, plus additions and minus losses during the cropping season. The additions include those from nitrogen fixation, where this occurs (see Chapter 13), mineralization of soil organic matter (see below), release from crop and tree residues (see Chapter 6), atmospheric deposition (see Chapter 9) and fertilizer application. Losses occur through nitrate leaching (see Chapter 7), volatilization and

denitrification. The present section concentrates on the quantification of the different forms of nitrogen in the soil and nitrogen mineralization. More detailed information on the other processes involved in nitrogen cycling in agroecosystems are given in the previously indicated sections of this book and in reference texts such as Stevenson and Cole (1999) and Havlin *et al.* (1999). A recent review of nitrogen management in African farming systems has been provided by Giller *et al.* (1997). Methods for using ^{15}N to track nitrogen movements in the plant–soil system are discussed in Sections 7.3 and 8.2.

Total nitrogen

The methods for the measurement of total nitrogen in soil and plant materials are based either on wet oxidation or dry combustion. The dry combustion method is used in automated CN-analysers (see also Section 4.2). Their advantage is the inclusion of all mineral and organic nitrogen forms and the simultaneous measurement of carbon and sometimes other elements. Because of the small sample sizes for certain instrument types, careful homogenization and fine grinding of the soil is necessary. For ^{15}N studies, CN-analysers can be directly connected to an isotope mass spectrometer (McGill and Figueiredo, 1993). The commonly used wet oxidation method is based on the Kjeldahl digestion procedure with sulphuric acid and a catalyst (Anderson and Ingram, 1993). A problem with this method is that some, but not all of the nitrate in soil is included in the measurement, which precludes the separate quantification of nitrate and addition of the value to the total nitrogen value from the Kjeldahl analysis. For soils containing relevant amounts of nitrate and nitrite, pretreatments are available that first convert nitrite into nitrate using permanganate and then nitrate into ammonium using reduced iron, which is included in the Kjeldahl analysis. These pretreatments are also recommended in studies with ^{15}N tracers because of the influence of highly labelled nitrate and nitrite on the tracer analyses (McGill and Figueiredo, 1993).

Mineral nitrogen

At the beginning of a cropping season, the soil may contain significant amounts of mineral nitrogen, including residual nitrogen from the previous crop and nitrogen that was mineralized between the two crops (e.g. during the dry season and especially upon rewetting of the soil at the beginning of the rainy season). Mineral nitrogen in the soil is usually measured by extracting field-moist soil samples with a 1 M or 2 M solution

of potassium chloride and colorimetric measurement of ammonium and nitrate (+ nitrite) in the extract, with the common assumption that nitrite is of little importance in well-aerated soils. A detailed procedure is given by Maynard and Kalra (1993).

Preseason mineral nitrogen can make a substantial contribution to the total nitrogen supply of a crop. In experiments with legume tree fallows on a sandy, nitrogen-deficient soil in Zambia, it was the most sensitive measure of plant-available nitrogen and explained close to 50% of the variance in crop yields (Barrios *et al.*, 1998). Soil nitrate alone has been widely used as a measure of nitrogen availability in temperate soils because it correlates with crop yield, is strongly influenced by soil management and has good reproducibility (Stanford, 1982). However, in tropical soils with a pronounced dry period, ammonium can be seasonally important because it can accumulate during the dry season (Wong and Nortcliff, 1995). Its inclusion in measurements of mineral nitrogen in tropical soils is generally recommended, unless it has been established that ammonium concentrations are small and more or less constant in a given soil. Mineral nitrogen accumulation in the soil as related to nitrogen leaching and recycling by trees is discussed in Box 8.1.

Nitrogen mineralization

Topsoils may contain several thousand kilograms of nitrogen per hectare, most of which is locked up in soil organic matter and not directly available to plants. The microbial transformation of organic into mineral nitrogen, or nitrogen mineralization, is a process of fundamental importance for the nitrogen supply in both natural and agricultural ecosystems. Net nitrogen mineralization is the difference between the total (or gross) nitrogen mineralization through microbial decomposition of organic matter and the simultaneous immobilization of nitrogen in microbial biomass. Gross rates of nitrogen mineralization can be determined with ^{15}N techniques (Barraclough, 1995). In the following discussion, nitrogen mineralization is taken to mean net mineralization.

Differences in the quantity and quality of soil organic nitrogen, climatic factors such as temperature and moisture, and soil management such as tillage and incorporation of organic matter, lead to considerable spatial and temporal variability in nitrogen mineralization rates in the field. Nitrogen mineralization can be measured in the field or under standardized conditions in the laboratory, or it can be predicted using models of variable complexity from knowledge of the soil type, climate and management. The prediction of nitrogen mineralization in the field is an important research issue in temperate, high-input agriculture because of the danger of nitrate leaching into the groundwater if the

supply of soluble nitrogen exceeds crop requirements (Campbell *et al.*, 1995).

Of particular interest for tropical agroforestry research is the measurement of nitrogen mineralization rates as related to soil management practices such as biomass application, fallow type and length, and method of soil tillage (Barrios *et al.*, 1996). The analysis of temporal and spatial patterns of nitrogen mineralization can provide important information for optimizing the spatiotemporal correspondence between nitrogen supply in the soil and its uptake by plants, for example through early sowing in soils with a large mineralization flush at the beginning of the rainy season, fertilizer application during periods with insufficient mineralization, or increased planting density in locations with high mineralization rates. The concept of synchrony and synlocation between nutrient supply and demand is further discussed in Section 6.1. Small-scale patterns of nitrogen mineralization within agroforestry plots can also be used as a sensitive measure for the effect of different plant species and management factors on nitrogen availability (Schroth *et al.*, 2000a, 2001c).

Nitrogen mineralization is measured in the field by extracting the mineral nitrogen (ammonium and nitrate) both at the beginning and at the end of an incubation period. Ideally, this is done with undisturbed soil, as soil disturbance may increase mineralization rates (Stenger *et al.*, 1995). Following a procedure devised and tested by Raison *et al.* (1987), Anderson and Ingram (1993) recommended the use of iron or plastic tubes for isolating a certain soil volume and preventing uptake of mineral nitrogen by roots. The tubes are driven into the soil and are covered to prevent leaching of mineral nitrogen by rain. For every site or treatment, three tubes are removed at the beginning and three other tubes at the end of the incubation (after about 1–2 weeks), and mineral nitrogen is extracted from the bulked replicates. Variants of the method involve placing resin bags at the top and bottom of the tubes in order to remove mineral nitrogen from rainwater and percolating soil solution (Zou *et al.*, 1992), or placing the undisturbed soil samples in sealed plastic bags, the buried bag method (Zou *et al.*, 1992; Barrios and Herrera, 1994; Subler *et al.*, 1995).

As cut-off roots cannot be removed before incubating undisturbed samples, errors in nitrogen mineralization measurements could occur because coarse woody roots continue taking up nitrogen, or because nitrogen is immobilized during microbial decomposition of root tissue (Raison *et al.*, 1987). Bauhus (1998) did not find indications for the latter process when using the method in a European beech forest (microbial biomass nitrogen was not increased in the incubated samples), although consistent negative mineralization rates in pasture in Brazil and woody vegetation in Zimbabwe indicate that microbial immobilization of nitrogen is a problem where large amounts of poor-quality organic matter are present (K.E. Giller, unpublished data).

The variability of net nitrogen mineralization rates measured in undisturbed soil cores is often large (Raison *et al.*, 1987). Using disturbed samples for the incubation can reduce the variability, because soil from several sampling positions can be mixed, and the sample for the determination of the initial mineral nitrogen content of the soil can be taken directly from the soil sample to be incubated. Coarser roots can be removed from the soil before the incubation. Plastic bags have been widely used for nitrogen mineralization experiments with disturbed soil. This is also called the buried bag method in the literature, so it is important to specify whether disturbed or undisturbed soil was used. If the method is used with disturbed soil, it is recommended that net nitrogen mineralization rates in disturbed soil are compared with those in undisturbed soil cores in preliminary experiments to ascertain the effect of disturbance on mineralization rates and to determine appropriate correction factors. In Amazonian forest soils, good agreement between nitrogen mineralization rates in disturbed and undisturbed soil samples has been found (Piccolo *et al.*, 1994; Schroth *et al.*, 2001c), although the agreement was not as good in soil from pasture (Piccolo *et al.*, 1994). In certain situations, penetration of tree roots into the incubated plastic bags or perforation of the bags by soil fauna can cause problems. The former problem can be prevented by periodic trenching around the incubated bags, and the latter by placing the bags between sheets of mosquito netting.

Measurements of nitrogen mineralization under standardized conditions in the laboratory are used for relative comparisons between soils and treatments and to determine input parameters for predictive models of nitrogen mineralization in the field (e.g. Campbell *et al.*, 1995). Both aerobic and anaerobic incubation methods are available. Depending on the exact research objective, disturbed or undisturbed soil samples can be used (i.e. excluding or including interference from soil physical properties), and factors such as temperature and moisture can be varied as required. If the soils were dried prior to the incubation, a mineralization flush usually occurs upon rewetting, which should be quantified separately from the subsequent mineralization rate, as treatment effects may differ between these two phases of mineralization (Schroth *et al.*, 1995b). Laboratory incubations have been used to compare the nitrogen availability of different fallow soils and to identify those characteristics of fallow tree species that are associated with high nitrogen availability in the soil. In a sandy savanna soil with low total nitrogen content (0.7 g kg^{-1}) in Zambia, nitrogen mineralization in the soil was highest after nitrogen-fixing trees with a low (lignin + polyphenol):N ratio in the leaves, indicative of rapid decomposition of the biomass (Barrios *et al.*, 1997) (see also Section 6.1). In contrast, in a forest soil in Côte d'Ivoire with higher total nitrogen contents (2.3 g kg^{-1}), soil nitrogen mineralization and crop

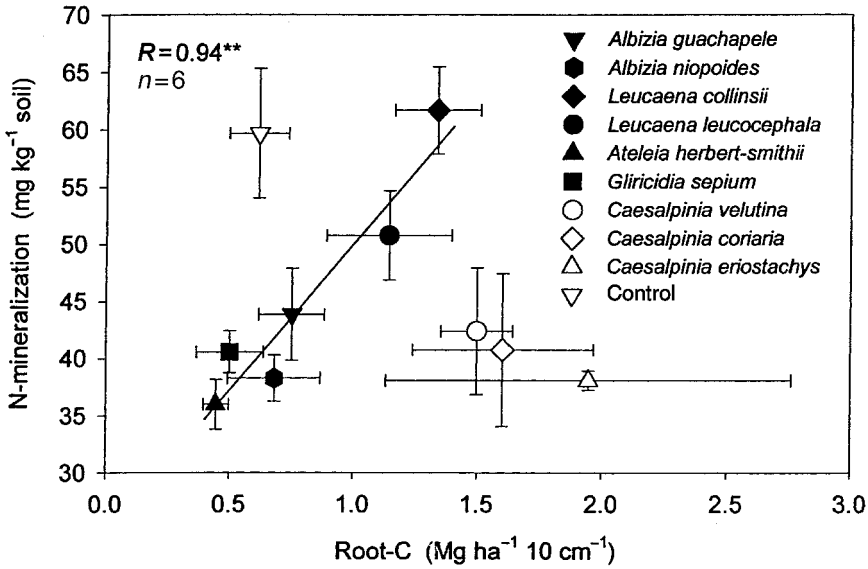


Fig. 5.2. Increase of nitrogen mineralization in the soil with increasing root mass of six nitrogen-fixing fallow tree species on a Lixisol in Côte d'Ivoire. The three *Caesalpinia* species and the spontaneous fallow (control) dominated by *Chromolaena odorata* were not nitrogen fixing (reproduced with permission from Schroth *et al.*, 1995b).

yields increased with litterfall and root mass of the fallow trees, but were not influenced by the ability of the trees to fix nitrogen (Figure 5.2). For detailed procedures of laboratory incubation methods see Anderson and Ingram (1993), Campbell *et al.* (1993) and Hart *et al.* (1994).

Fractionation of organic nitrogen: the potentially mineralizable pool

Considering the importance of nitrogen mineralization for plant growth and yields on most soils, it is not surprising that considerable efforts have been made to identify and quantify the labile, potentially mineralizable pool of soil organic nitrogen. On the one hand, these efforts aim at the development of easily measurable indices for the prediction of nitrogen mineralization in the field, which could replace lengthy incubation studies. On the other hand, an improved understanding of the physicochemical characteristics of labile soil nitrogen would facilitate the study of management effects on nitrogen availability and specifically the development of measures to increase the pool of readily available nitrogen in nitrogen-limited soils. The search for labile and stable pools of organic

nitrogen is also related to computer models designed to predict soil organic matter and nitrogen dynamics, which divide total soil organic matter or nitrogen into two or more pools with different turnover rates, but generally suffer from the inability to quantify these functional pools directly (see Box 4.1).

Physical fractionation methods

Several studies have shown that the addition of nitrogen-rich biomass to soil can lead to an increase of nitrogen in labile soil organic matter, and consequently increased nitrogen mineralization in the soil and nitrogen supply to crops (Barrios *et al.*, 1997). Depending on the soil and the quantity of biomass nitrogen applied, the effect may, however, become significant only after several years (Haggar *et al.*, 1993). In research with different planted tree fallows on a severely nitrogen-limited, sandy savanna soil in Zambia, the physical fractionation of soil organic matter according to particle size and density was found to be a sensitive indicator of the effects of nitrogen-rich biomass on nitrogen availability in the soil (for physical fractionation of soil organic matter see Section 4.3). Two- and 3-year-old planted tree fallows with *Sesbania sesban* and *Gliricidia sepium* increased the amount of nitrogen in the light particulate organic matter fraction (>150 μm, <1.13 g cm^{-3}), which correlated with nitrogen mineralization of the whole soil. Soil inorganic nitrogen and subsequent maize yields were consequently increased in these fallow treatments compared with continuous unfertilized maize (Barrios *et al.*, 1996, 1997).

In a study with ^{15}N-labelled tree residues on a Nigerian Lixisol, significant relationships between the uptake of residue-derived nitrogen by maize and residue-derived nitrogen in the particulate organic matter (>53 μm) also confirmed the value of this fraction as an indicator of high nitrogen availability (Vanlauwe *et al.*, 1998b). In a set of alley cropping trials in the West African moist savanna, the relative change in nitrogen in particulate organic matter was about twice the relative change in total soil nitrogen content, and the proportion of the total soil nitrogen in particulate organic matter increased significantly with annual nitrogen inputs in crop residues and tree prunings. This also suggests that nitrogen incorporated in this fraction is relatively labile and a more sensitive indicator of management effects than total soil nitrogen (Vanlauwe *et al.*, 1999).

Despite these promising results, it should be kept in mind that only part of the light fraction or particulate organic matter consists of readily mineralizable materials, another part consists of recalcitrant materials, whose relative contribution increases with progressive decomposition of added plant materials in the soil (Palm *et al.*, 2001; see Section 4.3). Also,

in many cases the light or particulate organic matter fraction is too small to account for all the readily mineralizable nitrogen in the soil (e.g. 2–3% of total soil nitrogen in the study by Barrios *et al.*, 1997) and it does not include labile pools such as microbial biomass which is mainly associated with clay-sized fractions (Palm *et al.*, 2001).

Chemical fractionation methods

These difficulties could possibly be overcome by combining physical fractionation methods with chemical indices of nitrogen availability. An approach to quantifying a labile fraction of soil organic matter through selective oxidation with permanganate has been discussed in Section 4.4. Acid permanganate has also been used to extract ammonium-N from soil through selective oxidation of soil organic matter (Stanford and Smith, 1978). The extracted amount was found to be closely correlated with potentially mineralizable nitrogen and thought to represent a fraction of soil nitrogen that is readily susceptible to biological mineralization. Gianello and Bremner (1986) found a close correlation between ammonium-N extracted by boiling the soil in 2 M KCl for 4 h and potentially mineralizable nitrogen for 33 Brazilian soils of widely differing clay and organic matter contents. Egoumenides (1990) separated the total nitrogen in a large number of tropical soils by acid hydrolysis (6 N HCl for 16 h) into a non-hydrolysable fraction (mainly phenol and quinone-amino acid complexes), a hydrolysable-distillable fraction (mainly hexosamines, amides and certain amino acids) and a hydrolysable-non-distillable fraction (mainly amino acids of microbial origin). Greenhouse and field studies showed that nitrogen is supplied to plants principally from the hydrolysable-non-distillable fraction, which decreases under cropping and is sensitive to management measures such as crop rotations and fallowing. The hydrolysable-distillable fraction, in contrast, is mainly influenced by soil conditions, such as clay content. Numerous other chemical methods for the assessment of labile organic nitrogen have been described (Keeney, 1982).

Microbial biomass nitrogen

The microbial biomass is another labile pool of soil organic matter and organic nitrogen as discussed in Chapter 4. However, nitrogen in microbial biomass is only sometimes correlated with nitrogen mineralization in the soil. This is not surprising, as the microbial nitrogen pool is too small to account for the amount of nitrogen mineralized from soil (Palm *et al.*, 2001), and mineralization of nitrogen from other organic materials results

from microbial activity rather than its biomass (Jenkinson, 1988a; Groot and Houba, 1995).

The high temporal variability of microbial biomass nitrogen requires attention when defining sampling plans, and repeated measurements during a cropping season may often be necessary to obtain a representative picture (Haggar *et al.*, 1993; Mazzarino *et al.*, 1993). Care is also needed in the selection of methods when attempting to relate microbial biomass nitrogen to nitrogen mineralization across different soils. In a comparison of different grassland soils, Hassink (1995) found that nitrogen mineralization in the soil increased with nitrogen in the total microbial biomass as measured with the fumigation–incubation method, but that the amount of nitrogen mineralized per unit of microbial biomass nitrogen was higher for sandy than for fine-textured soils. In contrast, the relationship between nitrogen mineralization and nitrogen in the 'active' microbial biomass as measured by substrate-induced respiration was independent of soil texture. This effect of soil texture, which was confirmed for other soils by Franzluebbers *et al.* (1996), was explained by differences in physical protection of soil organic matter in coarse- and fine-textured soils, and the second method was recommended for comparisons across different soil types. Detailed measurement procedures for microbial nitrogen are given in Anderson and Ingram (1993) and Voroney *et al.* (1993).

5.3 Methods for Soil Phosphorus (*J. Lehmann*)

Phosphorus availability is a limiting factor for plant production in many agricultural soils (Fairhurst *et al.*, 1999). This is especially true in the highly weathered soils of the humid tropics. At the same time, the global availability of phosphorus fertilizers is limited and known reserves may be exhausted in about 100 years with the current growth of phosphorus usage (Stevenson and Cole, 1999). In regions of the world without a history of use of phosphorus fertilizers, phosphorus deficiency is very common (Wild, 1988). A large portion of applied fertilizer phosphorus may be fixed to iron and aluminium oxides and is then not available for plant uptake. These facts make sound phosphorus management an imperative, especially in situations where funds for fertilizer purchases are limited, as in tropical smallholder agriculture. Agroforestry techniques can help to overcome some of these constraints (Buresh, 1999). However, because of generally low phosphorus concentrations in mulch materials (see Chapter 6), low atmospheric inputs (see Chapter 9) and low release by mineral weathering, adequate applications of phosphorus fertilizer are necessary in permanent agriculture to ensure economic and ecological sustainability (Buresh *et al.*, 1997; Newman, 1997). One-time replenishment of the soil

phosphorus capital, e.g. with phosphate rock, has been proposed as an option for soil fertility management in impoverished soils (von Uexküll, 1986; Buresh *et al.*, 1997).

In plants, phosphorus serves as a structural element in nucleic acids and plays an important role in energy transfer and other enzyme processes (Marschner, 1995). Common diagnostic properties of phosphorus deficiency are a darker green leaf colour due to higher chlorophyll contents (often with red pigments from anthocyanins), reduced leaf extension and a higher root-to-shoot ratio, since root growth is much less affected by phosphorus deficiency than shoot growth (Wild, 1988; Marschner, 1995). A high phosphorus supply is needed for nodulation of legumes and hence phosphorus deficiency can also seriously reduce biological nitrogen fixation (Marschner, 1995). The minimum phosphorus concentration required for nodulation of soybean was about 0.5 µg P l^{-1} in external solution (Marschner, 1995). For some trees suitable for agroforestry, however, no enhanced biological nitrogen fixation was noted with phosphorus applications on phosphorus-deficient soils (Reddel *et al.*, 1997; see also Section 13.1).

In contrast to nitrogen, soil phosphorus is almost entirely derived from primary minerals, mainly apatite. Inorganic phosphorus is present in the soil solution as phosphate ($H_2PO_4^-/HPO_4^{2-}$). It adsorbs to clay mineral surfaces by ligand exchange and by specific bonding (bridging ligands which form two ligand exchanges) or precipitates with calcium and aluminium depending on the soil reaction (Mott, 1988). The pH for optimum phosphorus availability ranges from 5.5 to 6.5 when determined in water.

The results of phosphorus analyses are greatly affected by the point of sampling, both vertically and horizontally. Inorganic phosphorus is very immobile in soil and small differences in sampling depth may yield completely different soil phosphorus contents. Vegetation effects (Tiessen *et al.*, 1999) and application of organic or inorganic phosphorus sources to certain crops during previous cropping seasons (Woomer *et al.*, 1998) cause uneven soil phosphorus distribution at a field and farm scale. This spatial variability creates challenges for scientific experimentation (Buresh, 1999).

Standard extraction procedures

Extraction procedures for plant-available soil phosphorus use bicarbonate (Olsen), acid ammonium fluoride (Bray 1 and 2), hydrochloric and sulphuric acid (Mehlich 1, 'double acid'), acetic acid and ammonium fluoride (Mehlich 2 and 3), ammonium carbonate and diethylenetriamine penta-acetic acid (DTPA) (Fixen and Grove, 1990), or anion exchange

resins (van Raij *et al.*, 1986). Inorganic phosphorus in the extract is most commonly analysed with the molybdate-ascorbic acid (molybdene-blue) procedure of Murphy and Riley (1962). Inorganic phosphorus yields may be affected by soil pretreatment: various studies have shown that air-drying of soil samples may lead to considerably higher (14–184%) extractable inorganic phosphorus due to liberation of phosphorus from microbial biomass (Sparling *et al.*, 1985; Srivastava, 1998). Summaries of soil test methods for plant-available phosphorus and their interpretation have been provided by Olsen and Sommers (1982), Novais and Smyth (1999), Fixen and Grove (1990) and several chapters in Carter (1993).

Adsorption experiments

In phosphorus-fixing soils, phosphorus fertilizer may be rapidly transformed into unavailable forms. The extent to which this happens depends on soil mineralogical properties (e.g. clay and especially allophane content and ferruginous nodules; Tiessen *et al.*, 1991), but also on the history of phosphorus applications and plant uptake. Two adjacent fields may have the same available phosphorus contents measured with a standard procedure, but respond completely differently to mineral phosphorus applications because their adsorption sites for phosphorus are saturated to a different degree. Adsorption experiments are a means of predicting the magnitude of fixation from applied inorganic phosphorus. Soil is equilibrated with different solutions containing increasing phosphorus concentrations, and adsorption isotherms are determined from the relation between adsorbed and dissolved phosphorus (Fox and Kamprath, 1970). From the proportion of immobilized to added phosphorus, the fertilizer quantity that is needed to increase the concentration in solution to the desired level can be calculated (Bache and Williams, 1971; Holford, 1979; Haggar *et al.*, 1991). By determining adsorption isotherms, it was shown that applications of organic matter decreased phosphorus adsorption due to competition for adsorption sites (Iyamuremye and Dick, 1996). Hue (1991) showed that organic acids decreased phosphorus sorption, but more so when phosphorus was added to the soil after the acids than when the phosphorus was added first. Accordingly, adsorption did not decrease if soluble phosphorus fertilizer (triple superphosphate) was added together with mulch on an Alfisol from East Africa (Nziguheba *et al.*, 1998). The extent of the reduction of phosphorus sorption may depend not only on the type of organic acids (Hue, 1991), but also on the phosphorus content of the applied organic matter (Singh and Jones, 1976). Recent greenhouse studies have shown that high calcium contents of leaf mulch may decrease the solubility of phosphate rock. Consequently, phosphate rock did not increase maize

growth when it was applied together with mulch (Smithson, 1999). In contrast, the dissolution of phosphate rock was increased by farmyard manure (Ikerra *et al.*, 1994).

Sequential fractionation methods

A central goal of some agroforestry practices is to increase nutrient efficiency by keeping applied nutrients in available form or to make recalcitrant nutrients available (Sanchez, 1995). Application of organic

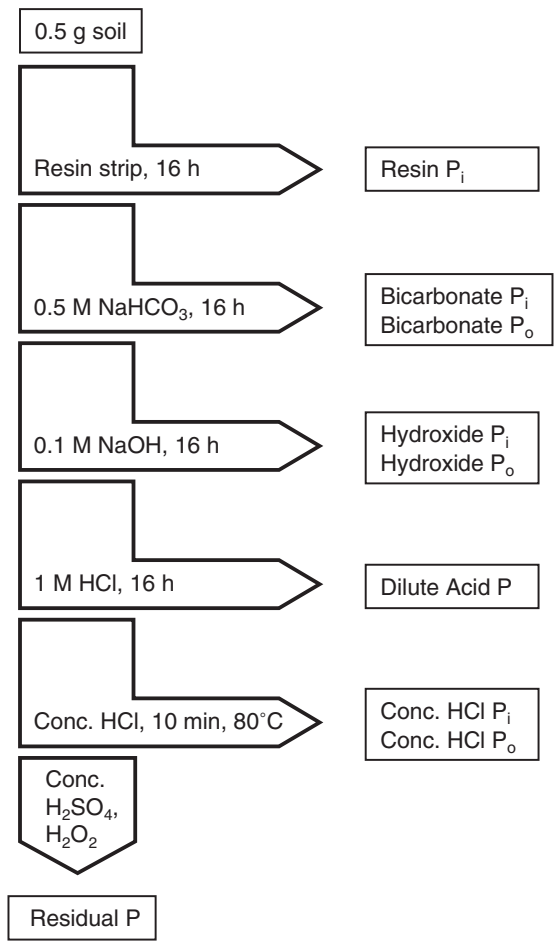


Fig. 5.3. Flow chart of the sequential phosphorus extraction method (after Tiessen and Moir, 1993). P_i, inorganic phosphorus; P_o, organic phosphorus; conc., concentrated.

Table 5.1. Schematic comparison of plant availability and characteristics of soil phosphorus pools obtained by different analytical methods.

Plant availability	Not available	Slowly available	Readily available	Immediately available
P pools	Occluded inorganic and long-term mineralizable organic	Adsorbed inorganic and medium-term mineralizable organic	Exchangeable inorganic and short-term mineralizable organic	Soluble inorganic and rapidly mineralizable organic
Soil solution extraction				inorganic/organic
Bray, Olsen, Mehlich		(inorganic)[a]	inorganic	inorganic
Isotopic exchange			inorganic	inorganic
Microbial biomass			organic[b]	organic
Sequential extraction	inorganic/organic	inorganic/organic	inorganic/organic	inorganic/organic
Physical fractionation	inorganic/organic	inorganic/organic	inorganic/organic	
NMR[c]	(inorganic/organic)	(inorganic/organic)	inorganic/organic	inorganic/organic

[a] Brackets indicate less information about the respective pool from the applied method.

[b] Only part of short-term mineralizable organic P.

[c] No differentiation according to plant availability possible; generally only NaOH-extractable P.

NMR, nuclear magnetic resonance.

nutrient sources such as compost, manure or mulch and the transformation of recalcitrant nutrient pools into more readily available organic nutrient sources are key aspects of agroforestry. Organic nutrient management also implies that changes in available phosphorus will not be adequately assessed by conventional extraction methods for inorganic phosphorus. Mineralization and immobilization processes, and consequently the flow from inorganic to organic phosphorus pools and vice versa, become more important. Therefore, a single analysis of available phosphorus will very often not be sufficient to estimate the ability of a soil to supply phosphorus to plants.

Sequential fractionation methods have been used for determining various soil organic and inorganic phosphorus pools with differing ecological properties. The Hedley fractionation method (Hedley *et al.*, 1982) has been widely adopted and latterly further modified (Fig. 5.3). The basic concept of the method is to sequentially extract several soil phosphorus compounds from the same soil sample by using extractants with increasing strength. The fractions that are extracted first have high plant availability, whereas the fractions that are extracted last have very low plant availability (Table 5.1). Organic phosphorus is determined by subtraction of inorganic phosphorus (determined after precipitation of organic matter) from total phosphorus (determined in digested samples; Tiessen and Moir, 1993). Bicarbonate and hydroxide extractions can also be carried out on separate subsamples to avoid phosphorus losses during the sequential soil treatment (Nziguheba *et al.*, 1998; Mutuo *et al.*, 1999). Soluble organic phosphorus is usually not determined by resin extraction, but may be an important pool and can be obtained from soil solution extraction (see Section 7.2). Bowman *et al.* (1998) suggested a more rapid method for analysing occluded and resistant soil phosphorus by subtracting acid-extractable phosphorus in an ignited sample from total soil phosphorus. A single-step method for determining organic soil phosphorus using concentrated sulphuric acid was found suitable for Nigerian savanna soils (Agbenin *et al.*, 1999).

With sequential fractionation it has been possible to assess the distribution of applied phosphorus in the soil profile (Beck and Sanchez, 1996), the proportion of biological to geochemical phosphorus (Cross and Schlesinger, 1995), the soil incorporation of phosphorus from mulch or manure (Ikerra *et al.*, 1994; Iyamuremye *et al.*, 1996; Nziguheba *et al.*, 1998; Solomon and Lehmann, 2000), the effects of burning (Ball-Coelho *et al.*, 1993) and tree effects on soil organic phosphorus (Tiessen *et al.*, 1992, 1999; Solomon and Lehmann, 2000; Lehmann *et al.*, 2001c).

Tree-specific phosphorus changes, especially in the dilute acid and organic phosphorus fractions, were found in a multistrata agroforestry system on an Oxisol in central Amazonia (Lehmann *et al.*, 2001c), whereas rice monocropping, legume pasture and native savanna affected inorganic

phosphorus in the bicarbonate and hydroxide pools of a Colombian Oxisol (Friesen *et al.*, 1997). The inorganic as well as organic bicarbonate and hydroxide phosphorus fractions did not show fertilizer effects as clearly as resin phosphorus in a tropical Alfisol (Mutuo *et al.*, 1999). An equilibrium between soil fractions seems to exist which replenishes plant-available resin-P_i from more recalcitrant phosphorus forms (bicarbonate and hydroxide) upon phosphorus uptake by plants (Schmidt *et al.*, 1997). With phosphate rock applications, a combined anion–cation resin gives a better measure of phosphorus availability than an anion resin alone, because it binds soluble calcium, thereby increasing phosphate solubility (Saggar *et al.*, 1992). In shifting cultivation in north-eastern Brazil, decline of phosphorus fertility resulted from mineralization of organic phosphorus and subsequent fixation to the mineral soil matrix rather than from net phosphorus export (Tiessen *et al.*, 1992).

A problem with phosphorus fractionation is that the ecological functions of the different fractions are not clear-cut and may depend on soil properties and management. The first two steps of the Hedley fractionation, resin and bicarbonate extraction, are standard methods for plant-available phosphorus (see above). However, the more recalcitrant fractions can be difficult to interpret. For example, the exchange between occluded and adsorbed phosphorus (characterized by acid-extractable and residual phosphorus) on the one hand and plant-available phosphorus on the other hand depends on their strength of adsorption. Also, the phosphorus supply capacity from organic sources may not be adequately assessed with sequential fractionation, as the activity of acid or alkaline phosphatase in soil may be more sensitive. In soils with high mineralization capacity, Oberson *et al.* (1996) found higher acid phosphatase activity after application of organic materials than in the control, whereas bicarbonate P_o did not show any significant differences.

A method for the direct measurement of phosphorus mineralization has recently been developed using phosphorus isotopes (Oehl *et al.*, 2001), but has not yet been tested in tropical soils.

Microbial biomass phosphorus

Next to nitrogen, phosphorus is the most abundant nutrient in microbial biomass. It constitutes a highly active pool with large turnover rates and short-term mineralizability (Brookes *et al.*, 1984) (Table 5.1). The analysis follows the fumigation procedure using bicarbonate extraction as described by Brookes *et al.* (1982). The extraction of phosphorus from fumigated soil with strips of anion exchange membranes was found to be more rapid and accurate than that with bicarbonate, especially in strongly phosphorus-fixing soils (Saggar *et al.*, 1990; Kuono *et al.*, 1995). The soil

water content prior to the extraction has an important impact on microbial phosphorus results and needs to be monitored (Sparling and West, 1989). Microbial phosphorus was found to be the second most sensitive indicator of soil phosphorus differences between intensive fallow and maize monoculture after phosphorus in the light fraction of soil organic matter (Maroko *et al.*, 1999). Effects of applications of *Tithonia diversifolia* mulch on microbial soil phosphorus were inconsistent between different studies from western Kenya. Small effects of organic phosphorus applications may not be readily detected in soil microbial phosphorus (Jama *et al.*, 2000).

Radioisotopes

Adsorption experiments can also be conducted with ^{32}P to determine isotopically exchangeable phosphorus (Fardeau *et al.*, 1996; Frossard and Sinaj, 1997). Whereas green manure did not affect phosphorus extracted by Bray's solutions or resin or phosphorus adsorption on an Oxisol in Brazil, it increased the amount of isotopically exchangeable phosphorus (LeMare *et al.*, 1987). Exchange processes between organic and inorganic soil phosphorus pools have also been studied with the radioisotopes ^{32}P and ^{33}P (Di *et al.*, 1997). The difference between exchangeable ^{32}P on a non-incubated soil (only physicochemical exchange) and the ^{32}P measured from incubated soils labelled with ^{32}P (physicochemical exchange plus phosphorus mineralization) gives an estimate of phosphorus mineralization (Lopez-Hernandez *et al.*, 1998). Isotopically labelled phosphorus fertilizer can also be used to follow its fate in soil phosphorus fractions (He and Zhu, 1997) or in the soil profile as affected by organic applications (Othieno, 1973). Additionally, the utilization of different soil phosphorus pools by different plant species may be estimated by ^{32}P labelling of the soil: plants utilizing unlabelled phosphorus are able to access phosphorus pools that are more recalcitrant than the isotopically exchangeable, soluble phosphorus fraction as shown for lupin (Braum and Helmke, 1995). For valid interpretation of radioisotope experiments, care has to be taken to meet underlying assumptions of isotope exchange kinetics (Frossard and Sinaj, 1997).

Other methods

From the total organic phosphorus in soil, no more than one-third has been chemically identified (Stevenson and Cole, 1999). Specific phosphorus compounds in soil comprise inositol phosphates, phospholipids, nucleic acids, phosphoproteins and metabolic phosphates

such as ATP. Organic and inorganic bonding of soil phosphorus can be analysed by nuclear magnetic resonance (^{31}P NMR) spectroscopy (Newman and Tate, 1980). With this method, increased amounts of labile organic phosphorus and, following mineralization, also of labile inorganic phosphorus were found after grassland conversion into conifer plantations (Condron *et al.*, 1996). Relatively labile diester-P compounds were shown to accumulate in soil from earthworm casts (Guggenberger *et al.*, 1996) and following application of manure (Solomon and Lehmann, 2000).

A combination of physical fractionation of soil (see Section 4.3) with phosphorus analysis can increase options for assessing transformation processes which are not detected by analyses of phosphorus in the bulk soil. This approach may, in many cases, be more useful than analysing organic phosphorus fractions on bulk soils, because it provides information on turnover and mineralizability of soil organic matter and therefore also of associated organic phosphorus (Tiessen *et al.*, 1999). Total phosphorus analyses, sequential phosphorus fractionation and ^{31}P NMR spectroscopy have been used for phosphorus determination in density and particle size separates (Maroko *et al.*, 1999; Solomon and Lehmann, 2000). Maroko *et al.* (1999) found that phosphorus effects of planted *Sesbania sesban* fallows and natural fallows, in comparison with continuous maize cropping, were most evident in the light organic matter fraction compared with a range of other phosphorus analyses.

Soil phosphorus availability for plants is influenced by the presence and activity of phosphorus-solubilizing bacteria in the rhizosphere, root release of protons and organic acids, mycorrhizal root infection, and root hair length and density. These factors are more important for the uptake of phosphorus than for that of any other macronutrient due to the low mobility of phosphorus in soil and its strong fixation to oxides. The ultimate test for soil phosphorus availability is plant uptake. Bioassays can give valuable information about the effects of different management interventions such as applications of mulch or mineral fertilizer on plant phosphorus uptake (Othieno, 1973; Haggar *et al.*, 1991; Jama *et al.*, 2000).

Phosphorus transfers in soil, e.g. from topsoil to subsoil, are seldom determined due to experimental problems of obtaining phosphorus from soil solution with ceramic suction cups or lysimeters (see Section 7.2). With little fertilization, amounts of inorganic phosphorus are usually low and possess low mobility especially in high phosphorus-fixing soil. In the absence of soil erosion, phosphate losses from agricultural soils do not usually exceed 0.5 kg ha^{-1} year^{-1} (Sharpley and Withers, 1994). However, organic phosphorus can be the dominant phosphorus compound in soil solution and dissolved organic carbon properties largely determine phosphorus mobility in soil (Qualls *et al.*, 1991; Donald *et al.*, 1993). Donald *et al.* (1993) demonstrated for a forest soil that 64% of the organic phosphorus was contained in the hydrophobic neutral fraction of dissolved

organic carbon that was only weakly adsorbed to the soil. Therefore, soil solution phosphorus can be mobile in its organic form (Schoenau and Bettany, 1987) and should be considered when calculating phosphorus budgets.

5.4 Methods for Soil Sulphur (*J. Lehmann*)

Arable soils of the humid tropics often have low total sulphur contents because of low contents of parent materials, strong weathering and high leaching losses. In comparison with other macronutrients, sulphur received little attention as a plant nutrient in tropical crop production in the past and few results have been published (Kang *et al.*, 1981; Acquaye and Kang, 1987; Motavalli *et al.*, 1993). Sulphur deficiency in tropical agroecosystems has recently increased, however, due to the more common use of nitrogen and phosphorus fertilizers with low sulphur contents (e.g. substitution of ammonium sulphate by urea and of single by triple superphosphate) as well as higher crop yields in some regions (Ceccotti *et al.*, 1998).

Up to 90% of sulphur in plants is present as amino acids and therefore bound in proteins. Sulphur is an essential element for many functions in plants (e.g. synthesis of chlorophyll) and sulphur deficiency not only decreases plant growth but also forage quality (Tisdale, 1977) and resistance to pests and diseases.

Visual diagnosis of sulphur deficiency in plants is difficult and criteria range from chlorosis in young leaves, starting from the edges, spoonlike deformations, succulence, poor pod formation, to stunted growth (Schnug and Haneklaus, 1998). Deficiency symptoms are more difficult to diagnose for monocotyledons than for dicotyledons and resemble those of nitrogen. Tissue analysis of total sulphur is a more reliable indicator of sulphur deficiency than visual diagnosis if the usual sampling criteria are followed, such as the collection of defined plant organs and development stages (Schnug and Haneklaus, 1998).

Sulphate-S

Plants take up sulphur in the form of SO_4^{2-}, which is the most common form of inorganic sulphur in agricultural soils. Grasses have a higher ability than legumes to utilize sulphate in soils (Havlin *et al.*, 1999), and excessive competition for sulphate-S in legume–non-legume mixtures may cause decreases in biological nitrogen fixation. Common extractants for plant-available sulphate-S include water, calcium chloride, sodium phosphate (Na_2HPO_4) (Kowalenko, 1993a), Na-acetate/acetic acid (Landon, 1991), lithium chloride (Tabatabai, 1982) and exchange resins (Searle, 1992).

However, the value of analysing sulphate-S for estimating the availability of soil sulphur for plants is not entirely clear (Schnug and Haneklaus, 1998) and additional availability tests are recommended.

Sulphate sorption characteristics are important for quantifying sulphur leaching and retention in tropical soils. Their determination is analogous to phosphorus sorption experiments (see Section 5.3). In soils high in clay, organic matter and exchangeable acidity, soil drying prior to analysis can result in large errors and experimentation with field moist samples is recommended (Comfort *et al.*, 1991). The extent of sulphate sorption in acid subsoils can differ from that of the topsoils as shown for an Alfisol and Oxisol from India (Patil *et al.*, 1989), but recycling by deep-rooting trees as occurs with nitrate (Box 8.1) has not yet been the subject of research. Adsorption generally increases with higher clay contents, oxide contents, and lower pH. Adsorption and precipitation of sulphate-S by carbonates has to be considered in certain dryland soils.

Mineralization of sulphur from soil organic matter and biomass

More than 95% of sulphur in soil occurs in an organic form. Since organic sulphur cannot be determined directly, total sulphur is usually measured using dry combustion and gas chromatography or dry ashing with alkaline oxidation followed by ion chromatography (Tabatabai *et al.*, 1988). Organic sulphur is calculated as the difference between total and inorganic sulphur. Sulphur availability for plants is closely linked to the mineralization of organic sulphur, similar to soil nitrogen (see Section 5.2). Sulphur mineralization can be measured by incubation methods. However, much higher mineralization rates of sulphur (but not nitrogen) have been measured when the mineralized sulphur was periodically leached out of the soil than in closed systems, indicating that certain incubation methods that are commonly used for nitrogen would lead to underestimation of sulphur mineralization rates (Maynard *et al.*, 1983). Therefore, mineralization studies are best conducted in open systems under field conditions (Eriksen *et al.*, 1998), where leaching of sulphate-S is allowed and mineralization processes are controlled by ambient temperature and moisture conditions. Plants have been shown to stimulate sulphur mineralization in the soil through a greater proliferation of microorganisms in the rhizosphere (Stevenson and Cole, 1999), which can make the prediction of sulphur availability more difficult when estimated from mineralization studies where roots are excluded.

The effect of sulphur from organic sources such as leaf mulch, animal manure or compost on sulphur availability depends on the concentration of sulphur in the organic materials. Sulphur immobilization in microbial biomass occurred after application of barley straw with low sulphur content

(2.2 mg g^{-1}), but not of rape leaves with high sulphur content (7.2 mg g^{-1}; Wu *et al.*, 1993). However, only a rough threshold value can be given for sulphur release from organic residues, which tend to mobilize sulphur if the C:S ratio is <200 and immobilizes it with a C:S ratio >400 (Stevenson and Cole, 1999). Sulphur release was found to be quite different from that of nitrogen, as soluble sulphate-S is initially solubilized in high amounts and sulphur mineralization is not closely related to the C:S ratio of the organic material (Janzen and Ellert, 1998). Short-term increases of available sulphur from applied animal manure can rarely be expected, although long-term accumulation of manure or urine in pasture soils increases soil organic sulphur (Saggar *et al.*, 1998) and leads to a higher supply of sulphate-S.

Fractionation of soil organic sulphur

Another approach to determining soil organic sulphur pools with different mineralizability is chemical fractionation. Ester-sulphate-S compounds can be analysed using reduction with hydriodic acid (HI) and subtracting inorganic sulphate-S. Carbon-bonded sulphur is then obtained by subtraction of ester-S from organic sulphur (Fig. 5.4). HI-reducible ester-sulphate-S is considered to be more labile than carbon-bonded sulphur, although some authors have reported the opposite (Janzen and Ellert, 1998). Carbon-bonded sulphur was not affected by trees in a multistrata agroforestry system in the central Amazon, whereas ester-sulphate-S increased in soil underneath trees with large nutrient recycling (Lehmann *et al.*, 2001c). Solomon *et al.* (2001) reported that forest clearing and maize cultivation in the Ethiopian highlands reduced both the carbon-bonded and ester-sulphate-S, whereas, in plantations of tea and *Cupressus lusitanica*, only the carbon-bonded sulphur fraction decreased. In a simplified conceptual model, carbon-bonded sulphur is considered to be released by mineralization, which is controlled by the needs of soil microorganisms for organic carbon, whereas ester-sulphate-S is released by enzymatic hydrolysis and therefore controlled by the supply of sulphur (McGill and Cole, 1981; see also comments in Ghani *et al.*, 1992). This dichotomous cycling of sulphur also explains variable N:S ratios of mineralized organic matter. Usually, sulphur was found to be more stable in organic matter than nitrogen upon soil cultivation (Stevenson and Cole, 1999). The dynamics of sulphur fractions under long-term cultivation have also been studied in humin, humic and fulvic acids (Bettany *et al.*, 1980); however, the analysis of ester-sulphate-S and carbon-bonded sulphur in these extracts may be affected by hydrolysis of organic sulphur compounds during the extraction and may not yield reliable results (Freney, 1986).

The analysis of organic sulphur in soil physical fractions offers the

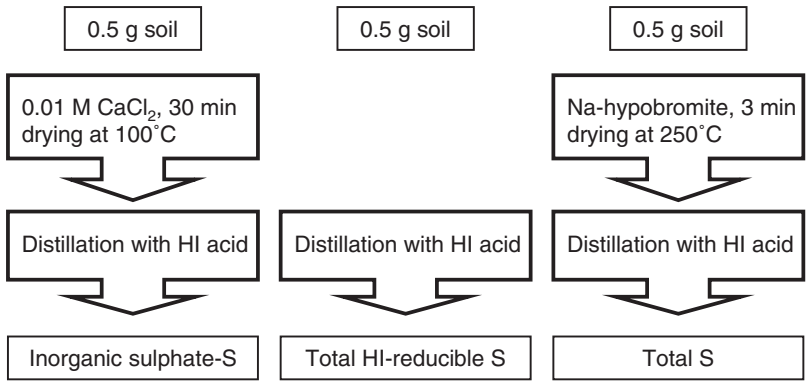

0.5 g soil
0.5 g soil
0.5 g soil
0.01 M $CaCl_2$, 30 min drying at 100°C
Na-hypobromite, 3 min drying at 250°C
Distillation with HI acid
Distillation with HI acid
Distillation with HI acid
Inorganic sulphate-S
Total HI-reducible S
Total S
Calculations:
Total S – Inorganic sulphate-S = Organic S
Total HI-reducible S – Inorganic sulphate-S = Ester-sulphate-S
Organic S – Ester-sulphate-S = Carbon-bonded S

Fig. 5.4. Conceptual outline of the sulphur fractionation according to Kowalenko (1993b). Total sulphur can also be obtained by dry combustion with an automated analyser.

possibility to detect tree effects on soil sulphur more sensitively and also to evaluate its stability after applications of mulch or animal manure. Sulphur can be analysed after fractionation according to particle or aggregate size and density (Anderson *et al.*, 1981). A fractionation method has been described which uses acetylacetone as extractant and different intensities of ultrasonic dispersion (Eriksen *et al.*, 1995).

Microbial sulphur comprises a pool of highly reactive sulphur in soils and is important for understanding the mineralization of carbon-bonded sulphur, although it only amounts to 1–2.5% of total soil sulphur. Microbial sulphur can be determined by the fumigation–extraction method using calcium chloride extraction (Saggar *et al.*, 1981; Wu *et al.*, 1994). Other soil organic sulphur measurements such as analyses of enzymes, amino acids and sulpholipids have been developed, but have not yet yielded easily interpretable results.

Other methods

Isotopic techniques using radioactive ^{35}S have been successfully employed to identify the fate of added sulphur in different soil pools and its subsequent mineralization (Freney *et al.*, 1971; Maynard *et al.*, 1983; Ghani *et al.*, 1993). Soil is incubated with carrier-free $^{35}SO_4^{2-}$ in the presence or

absence of carbon sources (glucose) and sulphate, and analysed for ^{35}S. Excess sulphate is leached and the soil is re-incubated. With this method, processes such as the incorporation of added sulphur from mulch or animal manure and the incorporation into soil sulphur pools such as ester-sulphate-S, carbon-bonded sulphur and sulphate-S can be studied.

When studying the sulphur balance of agricultural systems, exchanges of sulphur between the vegetation and the atmosphere have to be considered (Krouse *et al.*, 1991). If atmospheric sulphur concentrations are high, such as near the sea, sulphur uptake by plant leaves from the air can sometimes occur in sufficient amounts to meet plant requirements. On the other hand, plants may also release volatile sulphur compounds in response to nutritional or light stress. Sulphur losses from decomposing litter can be greater than from living plants (Janzen and Ellert, 1998).

5.5 Methods for Potassium, Calcium and Magnesium in Soil (*G. Schroth, J. Lehmann*)

The cations potassium, calcium and magnesium occur in several forms in the soil that differ in their availability to plants. The most readily plant-available fraction is that in the soil solution, followed by the exchangeable fraction, which replenishes the soil solution if nutrients are removed by either plant uptake or leaching. Potassium fixed in clay interlayers becomes available at a time scale from hours to weeks. The least available forms are various primary and secondary soil minerals, which release the respective nutrients upon weathering (Haby *et al.*, 1990). Together with sodium (where this is present at relevant quantities), potassium, calcium and magnesium make up the exchangeable bases of a soil. Numerous soil test methods are used for assessing the availability of these nutrients to plants, which are discussed in detail by Haby *et al.* (1990). As a rule, those methods that have been found most useful in the respective study region and for which locally calibrated reference values exist should be used. Of particular interest are multielement extractants such as Mehlich 3, which allows the simultaneous extraction of the exchangeable bases, phosphorus and various micronutrients (Tran and Simard, 1993).

Acid tropical soils, such as Oxisols and Ultisols, are characterized by low base saturation, and correspondingly low exchangeable contents of potassium, calcium and magnesium. Despite low contents, potassium availability rarely limits crop yields when acid soils are first taken into cultivation, although it becomes an important factor for permanent, intensive cropping once these soils have been limed and other deficiencies (e.g. phosphorus) have been corrected (von Uexküll, 1986). Small quantities of lime often increase potassium uptake by plants due to improved root growth, whereas larger quantities may depress potassium

uptake by increasing the cation exchange capacity associated with variable (pH-dependent) charges in the soil, thereby reducing potassium concentrations in the soil solution, and by antagonistic effects of calcium ions on potassium uptake. Low magnesium contents are common in acid soils, and magnesium deficiency can be induced by high aluminium contents (see Section 5.6) or by potassium fertilization. Tree crops tend to be more sensitive to magnesium deficiency than annual crops, and annual dicotyledons more sensitive than annual monocotyledons (von Uexküll, 1986).

With time under cropping the exchangeable soil contents especially of calcium and magnesium decrease and exchangeable acidity increases, unless cation losses caused by the exportation of harvested products, leaching and erosion are replaced by corresponding inputs of fertilizers and lime (Pieri, 1989; Juo *et al.*, 1995; Smyth and Cassel, 1995). In most soils, calcium and magnesium are more susceptible to leaching than potassium (see Section 7.1). If land is left to regenerate under spontaneous or planted fallow vegetation, losses of calcium (and probably other cations) in the plant–soil system may continue during the first years before nutrients start to accumulate due to atmospheric inputs, mineral weathering (where weatherable minerals are still present) and uptake from the subsoil (Szott *et al.*, 1999).

Tree species differ in their effects on nutrient cations in the soil, and such species-related differences can even be detected within closed forest stands (Finzi *et al.*, 1998). Trees are expected to reduce nutrient leaching from cropping systems (see Chapter 7), but they may also immobilize relevant quantities of nutrients in their biomass. In a long-term experiment, Hulugalle (1994) found lower exchangeable calcium contents in the soil under hedgerow intercropping than annual crops and pasture and explained this in terms of high calcium demand of the trees. Larger amounts of calcium and magnesium were immobilized in unpruned *Cordia alliodora* than in pruned *Erythrina poeppigiana* shade trees in coffee plantations (Beer *et al.*, 1998), and immobilization of potassium in the *Cordia alliodora* biomass was suspected to be a potential limiting factor to crop and tree productivity (Beer, 1988).

Some trees promote the accumulation of cations in the topsoil, presumably through efficient uptake by a large root system and subsequent release from litter. Reported cases include calcium accumulation by *Gmelina arborea* (Sanchez *et al.*, 1985; Fisher, 1995) and *Senna siamea* (Drechsel *et al.*, 1991). Elevated calcium contents also characterize the leaf litter of *Terminalia superba* (F. Bernhard-Reversat, unpublished data). Several tree species increased the calcium and potassium contents in the topsoil of a degraded rainforest site (Fisher, 1995). Relatively high potassium concentrations are found in the biomass of the large herbaceous plant, tithonia (*Tithonia diversifolia*), which is also rich in nitrogen and

phosphorus. The effectiveness with which this potassium was used by maize when supplied with tithonia biomass seemed to be comparable to the use of potassium from mineral fertilizer (Jama *et al.*, 2000). Increased potassium contents in the topsoil under certain cover crops have been explained with potassium redistribution from lower soil horizons (Smyth *et al.*, 1991; Barber and Navarro, 1994).

In accord with their different functions in the plant, potassium and calcium differ strongly in their release dynamics from both dead and living biomass. Potassium is rapidly leached from leaf and woody litter, whereas calcium often shows an absolute increase in biomass during the first stages of decomposition, possibly due to a combination of slow release and calcium import in fungal hyphae (Swift *et al.*, 1981; Maheswaran and Gunatilleke, 1988; Schroth *et al.*, 1992). Potassium is also leached in relatively large quantities from living plants. In studies in a rainforest in Borneo (Burghouts *et al.*, 1998) and in multistrata agroforestry and fallow in Amazonia (Schroth *et al.*, 2001a), potassium was the only macronutrient for which larger quantities were recycled from the standing biomass to the soil in throughfall and stemflow than in litterfall.

Cycling of calcium, magnesium and potassium in agroforestry systems can also be studied with tracers. Due to handling difficulties in conjunction with health hazards, radioisotopes (^{42}K, ^{28}Mg, ^{45}Ca) are only used in specialized experimentation under controlled conditions. However, subsurface applications of the elements lithium and rubidium (for potassium) and strontium (for calcium) can be used to assess root activity distribution (Fitter, 1986; van Rees and Comerford, 1986) (see Section 8.2). The determination of the fate of applied potassium and calcium fertilizer with these tracers may pose difficulties, because they would have to be applied at high concentrations, which would alter their behaviour in soil and during plant uptake.

5.6 Methods for Soil Acidity (*G. Schroth*)

Acid soils, especially Oxisols and Ultisols, occupy vast areas in the tropics. Naturally, these soils are mostly covered by forest or savanna, but during the last decades increasing areas have been cleared for agriculture. Despite the acidity and nutrient deficiency of these soils, their economic importance is substantial: 100% of the world tea, rubber, oil palm and cassava production and 90% of the world coffee production come from acid soils (von Uexküll and Mutert, 1995). However, the conversion of forest on acid soils for agricultural use very often leads to the development of unproductive, man-made savannas, such as the imperata grasslands of South-east Asia and some abandoned pastures of Amazonia. In the absence of the necessary inputs of nutrients and lime, the residual fertility from

the clearing of the vegetation is lost after only a few crop harvests, and the land is then commonly abandoned. Under conditions of increasing pressure on acid soils in the tropics from a growing population, the problems of soil acidity and infertility are intimately connected to losses in tropical forest area and biodiversity (von Uexküll and Mutert, 1995).

Acid soils require a humid climate for their development and are thus typically found in tropical savanna and rainforest zones. Under agricultural use, soil acidification is increased by leaching of basic cations and their removal with harvested crops; acidifying fertilizers such as urea, ammonium sulphate and potassium chloride; and the use of nitrogen-fixing legumes, for example, in pastures (Rowell, 1988; von Uexküll and Mutert, 1995). Agroforestry techniques may reduce problems of soil acidity by reducing leaching losses (see Chapter 7) and by increasing the level of soil organic matter, which detoxifies dissolved aluminium (see Chapter 4), although increased acidification due to the use of nitrogen-fixing trees may also be expected (van Miegroet and Cole, 1984).

Acid soils present a number of interrelated problems, including toxicity of aluminium, manganese and (under reducing conditions) iron as well as deficiencies of phosphorus, calcium, magnesium, potassium and micronutrients (e.g. molybdenum, boron). Low water-holding capacity (e.g. of Oxisols) and susceptibility to crusting, erosion and especially compaction (Oxisols, Ultisols) aggravate the problem (Marschner, 1995; von Uexküll and Mutert, 1995). Here, only aluminium toxicity as a key factor of acidity stress in many mineral soils is discussed. The risk of manganese toxicity is lower than that of aluminium toxicity in highly weathered tropical soils, because these often have low total manganese contents (Marschner, 1995). Mechanisms of plant adaptation to soil acidity have been discussed by Marschner (1995) and, specifically for tree species, by Fisher and Juo (1995). Lists of acid-tolerant and intolerant annual and perennial crop and pasture species and varieties are provided by Sanchez and Salinas (1981), and much useful information on tree species for acid soils can be found in the volume edited by Evans and Szott (1995).

Diagnosis of aluminium toxicity in plants

In soils with a pH in H_2O >5.5, aluminium is tightly held to the exchange surfaces, but as the pH falls below 5, exchangeable aluminium and aluminium concentrations in the soil solution increase markedly and can cause aluminium toxicity in sensitive plant species (von Uexküll, 1986). Neither visual diagnosis of shoots nor foliar analysis for aluminium is a suitable indicator of aluminium stress in cultivated plant species. Most of these are aluminium excluders and do not readily transport aluminium from the roots into the shoots, a notable exception being tea (*Camellia*

sinensis). In fact, aluminium concentrations in shoots of healthy plants may be higher than in those of aluminium-stressed plants. In tropical rainforests, aluminium includers and excluders exist at the same site, and these may vary in their foliar aluminium contents by two orders of magnitude. Aluminium concentrations in roots of aluminium-stressed plants are correlated with the severity of damage, but the analysis of roots for aluminium is difficult due to adhering mineral soil (Bergmann, 1988; Marschner, 1995).

Symptoms of aluminium toxicity first appear on the roots, which are shortened, thickened, show reduced branching and are brown to black in colour with black tips. Symptoms are limited to the actively growing tissue (Bergmann, 1988). Reduced root development, especially in the subsoil, makes plants sensitive to drought stress and reduces the access to nutrients in the subsoil, thereby also increasing nutrient leaching (Rowell, 1988). High aluminium concentrations in the solution impede the uptake of polyvalent cations such as calcium and magnesium and may thus induce deficiencies of these elements, whereas potassium uptake is usually unaffected (Marschner, 1995). Aluminium interacts with phosphorus availability through the reduction of root growth and by binding phosphate on root surfaces, cell walls and in the free space of plant roots. At higher aluminium concentrations, symptoms of aluminium toxicity may, therefore, be similar to those of phosphorus or calcium deficiency (Bergmann, 1988; Rowell, 1988).

Aluminium measurement in soil

To relate problems of aluminium toxicity and induced nutrient deficiencies in plants to soil properties, aluminium may either be measured in the dry soil or in the soil solution. The former approach is more compatible with standard soil sampling and laboratory practices and is, therefore, more common. Exchangeable acidity is usually measured by leaching a soil sample with 1 M KCl and quantification of the extracted acidity (aluminium and protons) by titration (Hendershot *et al.*, 1993b). Exchangeable acidity is given in $mmol_c\ kg^{-1}$ and/or as a percentage of the effective cation exchange capacity measured at the pH of the soil, e.g. by summing the exchangeable cations (Hendershot *et al.*, 1993a). In relatively aluminium-tolerant crop species and varieties, toxicity symptoms may appear at 60% aluminium saturation, and in less tolerant species and varieties at 30% aluminium saturation (von Uexküll, 1986). A procedure for fractionating aluminium forms in soils has been proposed by Soon (1993).

However, plants are directly influenced by aluminium in the soil solution, which is not always proportional to exchangeable aluminium. In

a comparison of the soil and soil solution chemistry of different land-use systems in central Amazonia, fallow and low-input agroforestry had more exchangeable acidity than high-input agroforestry and monoculture plantations, but the high-input systems had more aluminium in the soil solution due to the increased availability of fertilizer cations for exchange reactions with the sorbed aluminium and the acidifying effect of nitrification of fertilizer nitrogen. Peak concentrations of aluminium in the soil solution coincided with periods of highest nutrient availability following fertilizer applications and could have negatively affected the uptake of fertilizer nutrients (Schroth *et al.*, 2000b). Critical aluminium concentrations in the soil solution which negatively affect plant growth vary from about 1 mM for tolerant species to 1 μM for sensitive species (Rowell, 1988). In legumes, the critical concentration for nodulation may be substantially lower than that for the host plant (Ashwath *et al.*, 1995; Marschner, 1995).

The principal difficulty in interpreting aluminium concentrations in soil solution (but also exchangeable aluminium levels) is that, depending on the solution composition, different aluminium forms coexist which may differ widely in their phytotoxicity (Kinraide, 1991). Organic substances such as fulvic acids, humic acids and other organic acids may detoxify aluminium by forming complexes. Consequently, aluminium in the subsoil can be phytotoxic at lower concentrations in the soil solution than in the topsoil where organic matter contents are higher (Marschner, 1995), and the application of mulch and green manure can reduce aluminium toxicity in acid soils (Harper *et al.*, 1995). The concentration of different aluminium species in the soil solution can be predicted with models such as GEOCHEM, although not all the required stability constants may be accurately known (Kerven *et al.*, 1995). Also, the actual solution composition can differ from that predicted by equilibrium models during peak flow conditions after heavy rain when aluminium release may be influenced by diffusion processes (Franken *et al.*, 1995). Because of the suppressive effect of high aluminium concentrations on calcium and magnesium uptake, the molar Ca:Al and Mg:Al ratios in soil solution have been used as measures of aluminium-induced deficiencies of these elements and were superior in this respect to the concentrations of the individual elements (Marschner, 1995).

In addition to the effects of soil acidity on plants via toxicity and induced deficiency phenomena discussed above, soil acidity may also influence plant growth by affecting soil biological properties such as the abundance and composition of the soil fauna (Lavelle *et al.*, 1995) and plant diseases.

Methods of determining lime requirements for crops of differing sensitivity to soil acidity have been discussed by Sumner (1997) and van Raij and Quaggio (1997). For the assessment of plant litter effects on soil acidity, see Chapter 6.

Chapter 6
Decomposition and Nutrient Supply from Biomass

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6.1 Synopsis

One of the benefits that may be expected from agroforestry in comparison with annual and perennial monocultures is an increased production of biomass. When returned to the soil, this biomass protects the soil surface, releases nutrients, replenishes soil organic matter and provides carbon substrates for soil biota. This chapter focuses on above-ground biomass, which is easier to manage and which has been studied more intensively than root biomass in agroforestry, but the principles are also valid for the latter. Methods for studying root turnover as an important source of biomass input in the soil are discussed in Section 12.4.

With respect to nutrient inputs into the soil, there is a major difference between biomass grown *in situ* and fertilizers in so far as only part of the nutrients released from the biomass will be an external input, the remainder has been taken up from the same soil to which it is returned. Biomass transfer systems represent an intermediate situation in which the nutrients in the biomass are added to the site where the biomass is applied, but are removed from another site within the same landscape. Net nutrient additions in biomass to a system include biologically fixed nitrogen, nutrients taken up by the trees either from deeper subsoil horizons or from recalcitrant pools that would not have been readily available to crops. Nutrients taken up by extensive lateral tree roots beyond field boundaries are again an intermediate case. They can be seen as net additions to the system if they are taken up from a site that would have never been cropped (a river bed), but only as redistribution if they are taken up from a

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neighbouring field or from a fallow that will be cropped the following year, depending on the spatial and temporal scale under consideration. Other nutrients in biomass may not be additions, but rather 'avoided losses', such as nutrients taken up from the percolating soil solution that would have been leached in the absence of the trees (see Chapter 7).

However, a significant proportion of the nutrients in tree biomass may have been taken up in direct competition with crops. If tree biomass is applied to the soil, these nutrients are merely recycled. In simultaneous agroforestry systems, this may often be the main part of the nutrients contained in tree biomass. In contrast, in fallows, where direct tree–crop competition for nutrients does not occur or only between neighbouring crop and fallow plots, much of the nutrients in the tree biomass may consist of net additions and avoided losses.

The basic function of fallows is to produce biomass and thereby regenerate soil fertility. In the most widespread simultaneous agroforestry systems, the production of nutrient-rich biomass may also be an important function of the trees (e.g. pruned legume trees as shade in coffee and cocoa plantations), but it is usually not their only function and may often be of secondary importance to farmers in comparison with direct production functions (e.g. fruit or fodder trees in parkland systems in savannas). In hedgerow intercropping, the trees serve principally for the production of biomass and the recycling of nutrients through this biomass, but do not have direct production functions unless they are simultaneously used for fodder. The fact that this technique has not been widely adopted may indicate that these motives alone often do not provide sufficient incentive for farmers to plant and manage trees (Fujisaka, 1993). Agroforestry interventions need to be designed in the context of the niche within a farming system that the trees will fill. The optimization of carbon and nutrient supply will generally be constrained by farmer requirements for economic product, low competitiveness with crops and ease of management.

Nutrient cycling in biomass in agroforestry systems

The quantities of tree litter or prunings that are produced in agroforestry systems and the amounts of nutrients therein can be substantial. In coffee and cocoa plantations shaded by legume trees in Central America, 3–14 Mg ha^{-1} $year^{-1}$ of prunings are produced by the trees, containing 60–340 kg of nitrogen (Beer *et al.*, 1998). Leguminous trees in hedgerow intercropping produced up to 20 Mg ha^{-1} $year^{-1}$ of prunings, containing as much as 358 kg of nitrogen, 28 kg of phosphorus, 232 kg of potassium, 144 kg of calcium and 60 kg of magnesium (Palm, 1995). Very little is known about the amounts of carbon and nutrients released by root systems in agroforestry (see Section 12.4). Relevant nutrient release from roots

may occur when large amounts of superficial tree roots are destroyed by tillage at the beginning of a cropping season, and following the conversion of forest or fallow plots into crop fields.

The amount of biomass produced by agroforestry trees depends on the tree species, the number of trees per hectare, tree age, tree management and site factors. The nutrient concentrations in different parts of the biomass depend mainly on tree species, phenological stage (e.g. senescence, fruiting), management and site factors. Nitrogen-fixing trees normally have higher nitrogen concentrations in the biomass than non-fixing species, but this characteristic also varies widely between species (Palm, 1995). Deciduous species generally have higher nitrogen concentrations in the leaves than evergreen species (Eamus, 1999). Certain monocots such as bananas, bamboos and palms have relatively high potassium concentrations in their biomass (Sanchez *et al.*, 1985). Tree prunings normally have higher concentrations of mobile nutrients such as nitrogen, phosphorus, potassium and zinc than naturally fallen litter from which these elements are retranslocated by the tree prior to abcission (Marschner, 1995). Similarly, the young, leaf-rich biomass of frequently pruned trees has higher nutrient concentrations than the more woody biomass of infrequently pruned trees, although the quantity of biomass produced decreases with pruning frequency (Duguma *et al.*, 1988). Roots cut off during soil tillage would also be expected to have higher contents of certain nutrients than roots that die naturally, although the question of nutrient retranslocation from senescing roots requires further clarification (see Section 12.4). Trees will also have higher nutrient concentrations in their biomass when they grow in nutrient-rich than in nutrient-poor soil (Budelman, 1989; Palm, 1995). Compilations of nutrient concentrations in tree biomass can be found in Young (1997), Palm (1995), books on animal nutrition and in the Organic Resource Database developed by the Tropical Soil Biology and Fertility Programme (TSBF) and Wye College, London (www.wye.ac.uk/BioSciences/soil).

The amount of nutrients contained in tree prunings has often been compared with the nutrient requirements of the crops to which the prunings were applied. Such comparisons are useful to illustrate the relative importance of the nutrient fluxes through the tree biomass, but it must be kept in mind that in simultaneous agroforestry systems a large part of the added nutrients might have also been available to the crops in the absence of the trees. According to Palm (1995), the nutrients in 4 Mg ha^{-1} of leaves from any of four leguminous tree species could meet the requirements of a maize crop for nitrogen, calcium and much of the magnesium and potassium. Nitrogen was not supplied in sufficient quantity to meet crop demands by two non-leguminous trees, and phosphorus was not supplied in sufficient quantity by any of the tree species studied. This latter point is also valid for other annual crops

(Schroth *et al.*, 1995c). Biomass is usually a less efficient source of phosphorus than of other macronutrients.

Synchrony and synlocation of nutrient release with plant uptake

When biomass decomposes on or in the soil, the nutrients may either remain in the soil in mineral form, be incorporated in the soil biomass and soil organic matter (immobilization), be taken up by plants, or be lost from the system through leaching or in gaseous form. The relative importance of these different pathways depends on the respective nutrient, the decomposing material, and the biotic and abiotic conditions under which the decomposition process takes place. It has been hypothesized that the efficiency of the uptake of nutrients released from biomass by crops can be increased by improving the synchrony and synlocation (i.e. the temporal and spatial correspondence) of nutrient release with plant uptake. This concept can be applied to nutrient release both from biomass and from soil organic matter and is particularly relevant where losses of released nutrients are likely to be high, such as in humid tropical regions with high risk of leaching and denitrification during most of the year, or in savanna regions with a pronounced flush of nitrogen mineralization at the beginning of the rainy season (Myers *et al.*, 1994; Heal *et al.*, 1997).

Nutrient release from biomass and soil organic matter, and thus its synchrony and synlocation with crop uptake, can be influenced by management. Nutrient release from biomass can be controlled by selecting plant materials with desirable nutrient release kinetics and adjusting the timing and method of their application, with nutrient release being faster from soil-incorporated than from surface-applied materials. Nutrient release from soil organic matter is affected by the method and timing of soil tillage, mulching to reduce wetting and drying cycles, and the use of cover crops. To be effective in improving the uptake of a limiting nutrient by crops, it is important to consider whether synchrony and synlocation of release either directly from tree biomass or from soil organic matter is most relevant, so that appropriate management measures can be designed. This underlines the importance of studies of the fate of nutrients following their release from biomass.

When litter or mulch is exposed to rainfall on the soil surface, a soluble nutrient fraction is rapidly leached from the biomass into the soil either in mineral or organic form. This fraction includes most of the potassium, and for freshly cut (as opposed to naturally fallen) materials it may also include a relevant percentage of the phosphorus in the biomass (Babbar and Ewel, 1989; Schroth *et al.*, 1992). Rapid initial release of nitrogen from *Cajanus cajan* prunings has been attributed to leaching (Schroth *et al.*, 1992), but this has not been confirmed for other materials (Vanlauwe *et*

al., 1995). The fate of such rapidly released nutrients, and especially whether they are taken up by crops or washed further downward in the soil, should depend on the presence and activity of root systems in the soil at this time and on pedoclimatic conditions. Up to now such studies tracing the fate of released nutrients have concentrated largely on nitrogen, and little information is available on what happens to other nutrients including, for example, the relatively large quantities of potassium leached from tree litter or prunings.

The release of nitrogen from biomass and its fate in the soil–crop system has received considerable attention, both because of its importance to plant growth in many agroecosystems and the availability of suitable tracers which facilitate such research. Data compiled by Palm (1995) show that the recovery of biomass nitrogen by the crops to which it is applied is generally less than 20% and often closer to 10%. This compares with values of 50–70% for mineral fertilizers, although fertilizer use efficiency in Africa is often much lower, and 20–30% for manure (Finck, 1992; Giller and Cadisch, 1995). Studies with ^{15}N-labelled legume biomass have shown that most of the nitrogen not recovered in the crops is incorporated into soil organic matter, from which it is then successively released through mineralization (Ladd, 1981). Accordingly, additions of legume tree prunings during 8 years of hedgerow intercropping caused substantial increases in soil nitrogen mineralization rates in Costa Rica (Haggar *et al.*, 1993), and nitrogen mineralization rates in the soil were also substantially increased under a leguminous cover crop in an Amazonian agroforestry system (Schroth *et al.*, 2001c). Such results suggest that the nitrogen benefit to crops from biomass comes mainly from the longer-term build-up of readily mineralizable soil organic matter pools rather than from the short-term release of nitrogen from the decomposing material (Myers *et al.*, 1994; Palm, 1995). Consequently, it may be more important to improve synchrony and synlocation of nitrogen uptake by plants with its mineralization from soil organic matter than with its release from biomass, and this should be reflected by research priorities (see also Section 5.2). Little is known about the relative importance of short-term release as opposed to incorporation into mineralizable soil organic matter pools for phosphorus and sulphur (see Sections 5.3 and 5.4).

Prediction of nutrient release from biomass

As noted before, the dynamics of nutrient release from decomposing biomass under agroforestry conditions and the factors influencing it are the object of intensive studies. The principal objective of this research is to identify parameters that allow quantitative prediction of the time course of nutrient release from biomass as influenced by attributes of the biomass

itself (the resource quality) and the physicochemical environment in which it is decomposing. As a rule, the decomposition of organic materials in soil leads to an initial net mineralization of nitrogen if the C:N ratio is <20, and to an initial net immobilization of nitrogen through incorporation of nitrogen from the surrounding soil into the decomposer biomass if the C:N ratio is >30. During the decomposition process, carbon is released as carbon dioxide, and the C:N ratio is progressively reduced below the threshold value for net mineralization. For phosphorus and sulphur, the corresponding threshold values are C:P<200 and C:S<200 for initial net mineralization, and C:P>300 and C:S>400 for initial net immobilization. These values apply if carbon and the respective nutrient are in compounds with similar degradation rates (Stevenson and Cole, 1999).

For the prediction of nitrogen release from litter and prunings of agroforestry tree species, the C:N ratio alone has not been found satisfactory, as several other chemical characteristics of biomass affect the decomposition dynamics, especially their lignin and polyphenol content (Mafongoya *et al.*, 1998). Lignin, which is a low-quality substrate for decomposers, physically protects cellulose and other cell wall constituents, thereby reducing their degradation. A threshold level of 150 g kg^{-1} lignin has been suggested above which decomposition is impaired. Polyphenols include hydrolysable and condensed tannins. Insoluble condensed tannins bind to cell walls and proteins and make them physically or chemically less accessible to decomposers. Soluble condensed and hydrolysable tannins react with proteins and reduce their microbial degradation and thus nitrogen release (Mafongoya *et al.*, 1998). A critical soluble polyphenol content of 40 g kg^{-1} above which nitrogen release patterns are affected has been identified (Palm *et al.*, 2001). The relationship between polyphenol contents and nitrogen dynamics can be improved by measuring the protein-binding capacity of the polyphenols (Handayanto *et al.*, 1994). Both lignin monomer analogues and tannins have also been shown to directly inhibit the synthesis and activity of cellulolytic enzymes (Sinsabaugh *et al.*, 1991).

Several indices of mass loss and nitrogen release from biomass have been proposed. Of these the (lignin + polyphenol):N ratio has been suggested as the most robust for materials commonly used in agroforestry, although its application still suffers from methodological difficulties in the polyphenol measurements. Such indices are not constant for plant species, because nitrogen, lignin and polyphenol contents vary for the same species with plant part, age and growth conditions including soil fertility and water supply (Mafongoya *et al.*, 1998). Furthermore, there is evidence of rapid increases in tannin contents in the foliage of some trees following damage (Leng, 1997). For leguminous nodules, the ammonium and hexosamine-N contents were the best predictors of nitrogen release during decomposition (Wardle and Greenfield, 1991). Like the elemental contents

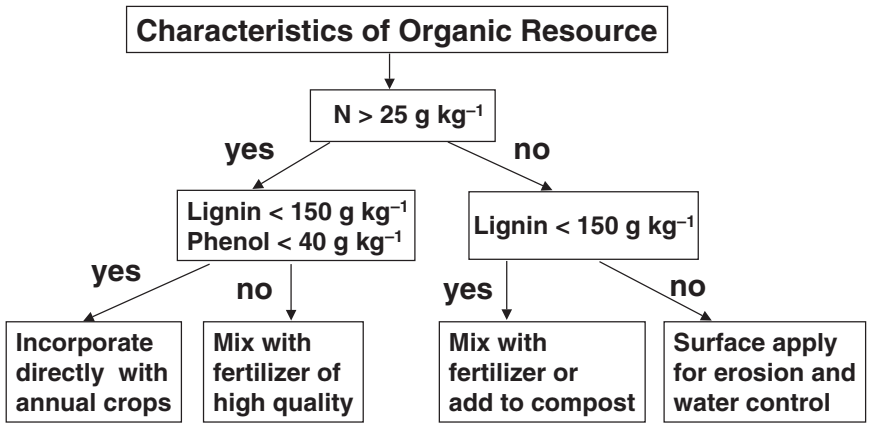


Fig. 6.1. 'Researcher's decision tree' for optimum use of biomass as a function of its quality (reproduced with permission from Palm *et al.,* 1997).

of biomass, the concentrations of organic constituents change during decomposition; for example, polyphenol contents of different litter types have been shown to decrease relatively rapidly (Pereira *et al.*, 1998). A decision tree for the optimum use of biomass as a function of nitrogen, lignin and polyphenol contents has been proposed, where high-quality materials are directly incorporated in the soil as a nutrient source for annual crops, while materials of intermediate quality are applied together with fertilizer or high-quality biomass or are composted, and slowly decomposing, nutrient-poor materials are applied to the soil surface for erosion control (Fig. 6.1). In the absence of chemical analyses, the quality characteristics can also be estimated from simple field tests (Fig. 6.2).

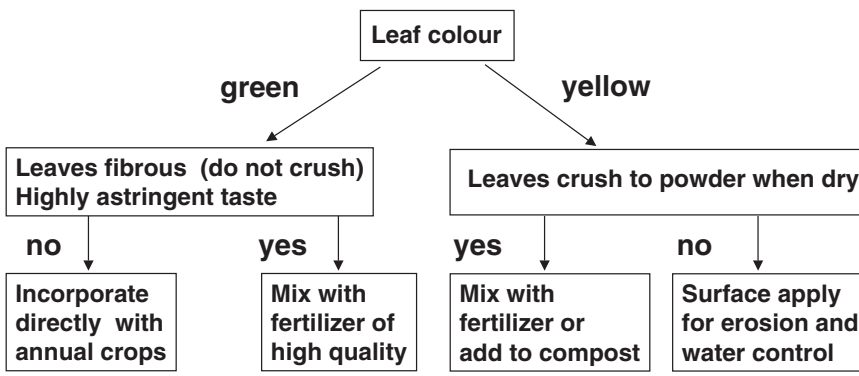


Fig. 6.2. 'Farmer's decision tree' for optimum use of biomass as a function of its quality (reproduced with permission from Giller, 2000).

A further potentially relevant measure of biomass quality is the soluble carbon content, which may be important for initial effects on microbial growth, and thus nutrient mineralization and immobilization. Its amount in plant materials is usually <15% (Palm and Rowland, 1997), but may be as high as 34% in foliage of *Eucalyptus* spp. (Bernhard-Reversat, 1993). In the latter work, mineralization rates were higher for soluble carbon from *Eucalyptus* spp. than from *Acacia mangium* foliage, indicating that effects of this carbon fraction on microbes may differ between species, depending on its chemical nature. Relationships between soluble and insoluble fractions of various litter types and carbon dioxide release during the initial stages of decomposition have been studied by Bernhard-Reversat (1998). The soluble carbon fraction in plant debris may also contain substances with phytotoxic and fungistatic properties (Ramamoorthy and Paliwal, 1993; Blum *et al.*, 1999).

Management of decomposition and nutrient release

There are several management options for manipulating decomposition and nutrient release patterns (Mafongoya *et al.*, 1998). These include the timing of biomass application and the incorporation of biomass into the soil, which accelerates decomposition, while surface application provides protection of the soil surface for longer. Of interest is also the possibility of mixing different litter types, such as recalcitrant materials, which provide a long-lasting surface mulch, with high-quality materials, which decompose and release nutrients rapidly. This can best be achieved by planting the respective plant species in association. Mixtures of litter types often have mineralization patterns equal to the weighted average of the patterns of the two separate materials (Palm *et al.*, 2001), but non-additive effects of biomass mixtures on decomposition and nutrient release rates have also been observed in many studies (Chapman *et al.*, 1988; Blair *et al.*, 1990; Briones and Ineson, 1996; Wardle *et al.*, 1997; Sakala *et al.*, 2000). The mixture of high-quality *Gliricidia sepium* prunings with prunings of *Peltophorum dasyrrachis*, which contains large concentrations of soluble polyphenols, led to complexation of proteins and unexpectedly low nitrogen release and nitrogen recovery by maize (Handayanto *et al.*, 1997). Mixing nitrogen-poor maize residues with senesced leaves of pigeonpea (*Cajanas cajan*) also resulted in more prolonged nitrogen immobilization than would have been expected from the individual components (Sakala *et al.*, 2000). Biomass may also be applied together with mineral nutrient sources to overcome nutrient imbalances (e.g. low phosphorus levels in biomass) and avoid initial immobilization of nutrients such as nitrogen and phosphorus when biomass with low concentrations of these elements (e.g. cereal straw) is applied to the soil (see Figs 6.1 and 6.2). The synergistic

effects of biomass and mineral fertilizers as nutrient sources have been reviewed by Palm *et al.* (1997).

Litter effects on soil acidity and nutrient mobility

The effect of different litter types on pH and nutrient mobility in acid soils has been the topic of recent studies (Noble and Randall, 1999). These authors found a highly significant relationship between the ash alkalinity of the plant materials and the pH shift of an acid soil during an 8-week incubation with ground leaves. Ash alkalinity was determined either by ashing the plant material followed by titration of the ash, or as the difference between the total cations and anions in the biomass. It was closely related to the calcium concentration in the samples, which could be used to estimate alkalinity. Based on ash alkalinity and the ability of the litter products to form stable organometallic complexes, which increase the mobility of nutrient cations in the soil, the authors suggested an index for the potential impact of plant materials on the base status of soils. Materials with:

- high alkalinity and high complexing ability will have a high risk of nutrient losses (but also potential for subsoil improvement);
- high alkalinity and low complexing ability are likely to increase the base status of the surface soil (the most favourable combination);
- low alkalinity and low complexing ability are unlikely to affect the soil; and
- low alkalinity and high complexing ability will have a strong podzolizing effect (the least favourable combination).

Litter effects on soil organisms

Besides the nutrient release from biomass, the microbial and faunal decomposer community has also been the object of studies which have the potential to contribute to improved management practices in agroforestry. The composition and activity of this community depends on characteristics of the biomass and environmental factors. The microbial decomposer community differs between surface-applied and incorporated crop residues, and this may affect carbon retention in soil organic matter (Holland and Coleman, 1987). The faunal communities in soil and litter of agroforestry systems are also influenced by the quantity and quality of litter and their small-scale distribution (see Chapter 16). Faunal communities not only may influence the path of decomposition and nutrient release (Tian *et al.*, 1995b), but may also affect soil properties such

as soil structure and soil organic matter dynamics. Future research in this area should focus on how biomass can be used strategically to improve soil conditions through the stimulation of beneficial faunal and microbial groups (see Chapter 16).

The animal pathway

Instead of being directly applied to the soil as a source of nutrients and organic carbon, tree prunings are also often of interest as protein-rich fodder for domestic livestock. The resulting farmyard manure may be used for fertilizing crops, thereby recycling part of the nutrients in the biomass. Many tropical crops respond to manure applications with increased yields (Webster and Wilson, 1980). Whether biomass is better applied to the soil or used as fodder depends on the materials in question and on the degree of integration of crop and livestock production in the farming system. Within the season of application, nitrogen-rich biomass from trees such as *Calliandra calothyrsus* will release more nitrogen when applied directly to the soil than would be released from the manure resulting from feeding it to ruminants. In contrast, processing materials with low nitrogen but high decomposable carbon contents, such as barley straw, through an animal may lead to lower nitrogen immobilization when the faeces are applied to the soil than would be the case with fresh plant material (Delve *et al.*, 2001). In a study in western Kenya it was concluded that feeding *Calliandra calothyrsus* biomass to diary cattle and using the manure for soil improvement was economically more advantageous than applying the biomass directly to a maize crop (Jama *et al.*, 1997).

The factors that determine the transformation of biomass into soil organic matter have been discussed in Chapter 4.

6.2 Methods for Biomass and Nutrient Input with Litter

Soils under agroforestry receive biomass inputs in the form of natural litterfall and prunings. The quantification of nutrients in prunings is straightforward. The material should be subdivided into more or less homogeneous groups with respect to nutrient concentrations and moisture content, such as leaves, small wood (usually <2 cm), larger branches, and flowers and fruits. These groups should be weighed, subsampled and analysed separately. Methods for quantification of biomass and nutrient inputs in litter have been described in detail by Anderson and Ingram (1993).

Litter traps

Tree and shrub litter is usually collected in litter traps, which may be constructed with plastic netting (mosquito netting is often used), mounted on a wooden or metal frame. A common collector size is between 0.25 and 1 m^2 (Anderson and Ingram, 1993). The collectors are placed at a few decimetres above the ground to avoid contamination of the litter by soil or animals. This also accelerates drying of the collected litter, thereby slowing down decomposition processes. The traps must allow drainage of rainwater, but the openings must be small enough to retain small leaflets and litter fragments (<1 mm). The leaflets of certain legume tree species are too small to be quantitatively retained by these commonly used collectors. In such cases, either tissues with smaller openings or a second layer of netting placed at an angle of 45° to the first can be used (F. Bernhard-Reversat, personal communication). Losses can be estimated, and eventually correction factors determined, by mounting the collector tissue on buckets or other closed recipients (Schroth *et al.*, 1995b).

Where the vegetation is more or less homogeneous and spatial patterns of litterfall are not of interest, the collectors can be placed randomly in the measurement plot. For obtaining a 5% standard error about the mean, 20 collectors or more per plot may be necessary (Anderson and Ingram, 1993). To improve the representativeness of the plot mean obtained (or to reduce the number of replicate samplers without losing precision), the positions can be changed from time to time as recommended for throughfall collectors (Lloyd and Marques, 1988). In studies where spatial variability of litter and nutrient inputs is of interest, permanent positions may be more useful.

Agroforestry plots are usually not homogeneous and so systematic or stratified random sampling designs are usually preferable to random designs in litterfall studies (see Section 3.4). A systematic design for a planted fallow with trees at 2 m × 2 m would be to place collectors of 1 m × 1 m size in a way that the sampling area covers one quarter of the space between four neighbouring trees with one corner of the collector attached to a tree (see discussion of smallest representative units in Section 3.4). For other planting designs, the collector form, size and location should be chosen so that the sampled area covers a representative section of the plot. Other agroforestry plots are best subdivided into more or less homogeneous subplots, in which collectors are placed in a random pattern (stratified random sampling). For isolated trees (or trees of a certain species within a multispecies canopy), samplers should be used that cover a sector of a circle with the tree in the centre and the outer limit of the collector reaching 1–2 m beyond the limits of the tree crown. Preferential wind directions should be considered when deciding about the sampling design. For large trees, the angle of the sector that is covered by the collector can

be reduced at some distance from the trunk to limit the total size of the collector. Small trees often produce only a small amount of litter, so to obtain reliable data it is often necessary to collect all of it (F. Bernhard-Reversat, personal communication).

Litter traps measure the litter input into the soil, which is not always the same as the litter production by the tree crowns covering this soil. Litter typically falls during windy weather, and the leaves of tall trees can travel considerable distances before they reach the ground (or the collector). In continuous vegetation, such as forests, this may not cause errors, but it can lead to underestimation of litter production in small, isolated experimental plots or from isolated trees in savannas, as some of the litter may fall too far from the tree to be collected. Also, litterfall measurements in one plot may be influenced by the litter of neighbouring plots.

Depending on the research question, the collected litter may have to be sorted according to species. In general, it should be separated into leaves, small woody litter (twigs <2 mm, bark), reproductive structures, and trash (sieve fraction <5 mm) (Anderson and Ingram, 1993). Subsamples should be dried at 60–80°C for the determination of dry weight and subsequent nutrient analyses. Wood samples may require drying at higher temperatures (105°C).

If nutrient analyses are intended, the litter should be collected from the traps at least every 2 weeks to reduce leaching and decomposition, although more frequent collections are recommended for litter types that decompose rapidly and under high-rainfall conditions. Under dry conditions, less frequent collections may be sufficient, although contamination by dust or animals may occur (Anderson and Ingram, 1993). When comparing nutrient concentration data from different litter studies, the collection interval and resulting experimental errors may have to be taken into account (Cuevas and Medina, 1986).

Other methods

Quantification of coarse litter such as branches or palm fronds requires larger sampling areas. As contamination with soil and decomposition losses are less of a problem than with leaf litter, the sampling area can be marked on the ground without recourse to a collecting device. The concept of smallest representative unit of a plot is useful for this (see Section 3.4). Litter fractions that are measured in the small collectors should be removed from the coarse litter before weighing. The litter is then also subsampled for the determination of dry weight (Anderson and Ingram, 1993).

The litter production of herbaceous vegetation such as grassland is more difficult to measure than that of trees, because collectors cannot be

installed. A suitable method for measuring litter production and disappearance is the paired plot technique (Moore and Chapman, 1986).

Biomass and nutrient inputs with litterfall and prunings in various production systems in the humid tropics have been reviewed by Szott *et al.* (1991).

6.3 Methods for Decomposition and Nutrient Release from Biomass

The processes of litter decomposition and associated nutrient release, either under agroforestry conditions or in natural tropical ecosystems, have attracted the attention of many researchers, and numerous discussions of methods and results are available in the literature. Recent work has concentrated particularly on chemical characteristics of the litter or biomass that influence decomposition and nutrient release, and on the effect of decomposer organisms and environmental factors on these processes. The principal difficulty in decomposition experiments is that the material has to be identified during the decomposition process with minimal disturbance to effects of environmental factors, such as microclimate or access of decomposers, on decomposition (Anderson and Ingram, 1993).

Litterbags

Decomposition of leaves and other materials of small size is usually studied in mesh bags with varying mesh sizes. Litterbags are made from plastic materials, but, where termites feed on the plastic or are to be excluded from the litter for experimental reasons, the mesh can be stainless steel or brass. The mesh size should be about 5–10 mm, which is small enough to minimize loss of fragments but large enough to allow access by invertebrates and to reduce alteration of the microclimate. Litterbags with finer mesh on their lower side and coarser mesh on their upper side are a good compromise, especially when small leguminous leaflets are under study (Lehmann *et al.*, 1995). Care should be taken that the litter is arranged in a similar way in the bag as in the natural litter layer to imitate its microclimatic conditions. Litterbags can be buried in the soil when the decomposition of ploughed-in materials is under study, or can be suspended at some distance above the soil for monitoring the decomposition of leaves of large prunings in a mulch layer that remain attached to the branch and decompose without having contact with the soil (Gupta and Singh, 1981).

Litterbaskets

Litterbaskets are an alternative to litterbags, and are supposed to create less artificial conditions than litterbags, while retaining more experimental control than working with unconfined litter (Blair *et al.*, 1991; Anderson and Ingram, 1993). They have a diameter and height of 1–3 dm and are made of metal or plastic mesh. The baskets are partly buried in the soil and can be filled with either disturbed soil, for arable conditions, or undisturbed soil, for uncultivated soils. The organic material whose decomposition is to be studied may be placed in the baskets either in layers for mulch or litter, or mixed with the soil for ploughed-in green manure, crop residues or roots. Processing the samples after the incubation is obviously more labour-intensive than with litterbags placed on the soil surface. The method also allows study of the effects of the decomposing litter on the soil (Lehmann *et al.*, 1998b).

Unconfined litter

For decomposition studies of larger woody materials, these can be marked or attached to a tether for easier localization and reweighed after certain time periods. For wood, the loss in density provides a means for monitoring the decomposition process with little disturbance to the sample and has been linearly correlated with carbon dioxide evolution (Yoneda *et al.*, 1977). In a study in the central Amazon, wood decomposition as measured from weight loss and respiration rate decreased with increasing wood density and increased with its moisture content (Chambers *et al.*, 2000, 2001).

Where litter is released in pulses and can be clearly distinguished on the soil from older litter, or where no older litter is present, decomposition can be monitored by periodically quantifying the litter lying on the soil. This method can be useful for studying decomposition of crop residues after the harvest, or in systems where mulch is applied at regular intervals as in hedgerow intercropping or in systems with regularly pruned shade trees. It should, however, be noted that weight loss of unconfined leaves is a measure of litter breakdown rather than of its mineralization (Woods and Raison, 1982). If it is only the effect of added biomass on the soil, but not the transformation of the biomass itself that is to be monitored, then the biomass can be mixed with soil, from which samples are periodically taken for analysis (e.g. of mineral nitrogen). Ground leaf material has been used in this type of experiment to study chemical leaf characteristics independently from their physical properties (Palm and Sanchez, 1991).

Root decomposition

In studies of root decomposition, the compromise between the avoidance of artificial conditions and the need to identify the decomposing material is particularly problematic. Unfortunately, accurate decomposition rates are of central importance for certain models for estimating root turnover (see Section 12.4). The most frequently used method for measuring root decomposition is the litterbag technique, although it seems to underestimate root decomposition rates in comparison with other methods. Beside the difficulty of collecting sufficient root material in the correct state of senescence and diameter class, the removal of rhizosphere constituents when preparing the roots for the incubation is a potential source of error (Vogt *et al.*, 1991). In any case, the roots in the litterbags should be mixed with soil to ensure a good root–soil contact.

As an alternative to litterbags, the decomposition of senesced roots of annual crops can be monitored *in situ* by sequential soil coring after the crop harvest, if weed growth is controlled or if dead weed roots can be distinguished from those of the crops (Schroth and Zech, 1995b; Lehmann and Zech, 1998). For perennial crops and trees, an equivalent situation is created with the trench plot technique. Soil monoliths, from which the vegetation is removed, are isolated from the surrounding soil, for example with plastic sheets, thereby cutting off roots in the monolith and impeding the ingrowth of new roots. Monoliths of variable sizes have been used, for example 1 m deep around a 1 m × 3 m plot (McClaugherty *et al.*, 1984), and 30 cm deep around a 20 cm × 20 cm plot (Santantonio and Grace, 1987). The decomposition of the roots in the monolith is monitored by sequential soil coring. The decomposition of tree roots in trench plots is generally about twice as fast as in litterbags. Problems of the trench plot technique include higher soil moisture compared with the surrounding soil (possibly increasing decomposition), and retarded decomposition of larger severed roots with significant carbohydrate reserves. There is also evidence that the presence of living roots may influence decomposition. It is not possible to decide which of the two methods produces more reliable results from present knowledge (Vogt *et al.*, 1991), and so the use of a combination of methods is advisable.

Technically more demanding than the aforementioned techniques are methods that depend on carbon isotopes for measuring the transfer of carbon from roots to soil, and thus root decomposition. This can be done after labelling roots with ^{14}C, either in the laboratory (Jones and Darrah, 1994) or in the field (Swinnen *et al.*, 1995), and measuring the $^{14}CO_2$ evolution from the roots or temporal changes of the distribution of the isotope between roots and soil (Cheshire and Mundie, 1990; Swinnen *et al.*, 1995). Alternatively, the input of root carbon from C_4 species in soil previously dominated by C_3 species (or the other way round) can be

quantified by comparing treatments where either root and shoot biomass or only root biomass is returned to the soil (Balesdent and Balabane, 1996). These techniques have not yet been used with trees, although they seem to be appropriate in principle. Minirhizotron observations have also been used to determine *in situ* root decomposition rates (see Section 12.4).

Litter selection and pretreatment

The litter for decomposition studies has to be chosen in agreement with the objectives of the experiment. Where the decomposition of prunings is to be assessed, fresh leaves should be used. Where the decomposition of naturally fallen litter is to be studied, freshly fallen leaves should be collected, which have lower contents of certain nutrients (nitrogen, phosphorus, potassium) and soluble organic carbon than green leaves and, therefore, decompose more slowly (Woods and Raison, 1982). Similar considerations apply to roots: for decomposition studies of roots that have been cut off during tillage, living roots can be collected, but, for natural root turnover, senescent roots would be required, which decompose at very different rates from severed living roots (Publicover and Vogt, 1993). Senesced roots are, however, difficult to collect in sufficient quantity. Drying of litter before the incubation should be avoided as this may introduce artefacts (Anderson and Ingram, 1993), unless the materials are also subjected to drying and rewetting under natural conditions for decomposition, which is often the case in the tropics.

Nutrient release from decomposing litter

Nutrient release from decomposing litter is usually measured by collecting litter samples from litterbags or tethered litter at intervals and then measuring their weight and nutrient concentration and expressing it relative to the initial values. Alternatively, nutrient release from undisturbed litter can be monitored by collecting the leachate beneath the litter with trays or funnels and subtracting the nutrient input in rainfall or throughfall (Woods and Raison, 1982). This approach allows separation of nutrients released in organic and inorganic forms, but does not take into account gaseous nutrient losses from decomposing biomass (Janzen and McGinn, 1991). The two approaches can also be used in combination. Tubes filled with mixtures of soil and litter which are periodically leached and the leachates analysed provide a means to study nutrient release and immobilization dynamics under controlled conditions with a high temporal resolution (Sakala *et al.*, 2000).

Nutrient release studies from decomposing roots are complicated by

the problem of soil contamination, which is greater for fine than for coarse roots and tends to increase as decomposition advances. According to Misra (1994), soil contamination can account for up to 28% of the potassium in a root sample. The amount of soil adhering to the roots can be estimated from ashing (Misra, 1994) or from the determination of total carbon in the sample, assuming that a soil-free root has a carbon content of about 45% (Schroth and Zech, 1995b). The nutrient concentration of the soil can be determined by digesting a separate soil sample with the same method used for the root samples. Misra (1994) also found that washing of roots resulted in potassium losses of 24% and that the nutrient losses further increased when the root samples were stored in water after separation from the soil. Similar rules presumably apply to other types of biomass that become contaminated with soil during decomposition studies. Minimum contact with water and immediate drying of samples after their separation from soil are generally recommended.

Particularly useful for agroforestry research are decomposition studies with ^{15}N-labelled biomass as these allow tracking of the incorporation of released nitrogen into vegetation (trees and crops) and different components of the soil organic matter (Haggar *et al.*, 1993; Xu *et al.*, 1993; Vanlauwe *et al.*, 1998a). For problems of pool substitution with ^{15}N-labelled biomass which can sometimes lead to large underestimates in nitrogen recovery see Section 7.3 and McDonagh *et al.* (1993).

Measuring the role of decomposer groups and environmental factors in decomposition

The contribution of different decomposer groups, such as microorganisms and invertebrates of different sizes, to litter decomposition can be assessed by using litterbags of different mesh sizes. For exclusion of all invertebrates, a mesh of 20–60 μm is required (Woods and Raison, 1982). The efficiency of exclusion should, however, be checked as invasion of 20 μm litterbags by microarthropods, presumably through oviposition or juvenile forms, has been reported (Vreeken-Buijs and Brussaard, 1996). Takamura and Kirton (1999) measured wood decomposition with an open tray design that they claimed excluded most termites while permitting access of most other invertebrates. Decomposer groups can also be excluded by adding biocides (such as naphthalene for invertebrates) to the incubated material (Heneghan *et al.*, 1999), but these may have side effects on non-targeted organisms (Blair *et al.*, 1991). Alternatively, certain decomposer groups can be selectively added to incubated litter to study their effect on litter breakdown (Tian *et al.*, 1995b). Woods and Raison (1982) proposed to separate total weight loss of unconfined litter into weight loss due to reduced leaf area (presumably caused by fragmentation or consumption

by larger invertebrates) and weight loss per unit leaf area (presumably caused by decomposition by microbes and microfauna). The use of enzymic techniques for the mechanistic study of litter decomposition processes has been reviewed by Sinsabaugh *et al.* (1991).

Monitoring environmental factors such as rainfall and moisture content of the litter during decomposition experiments may help to explain observed patterns of decomposition and nutrient release (Gupta and Singh, 1981; Reddy, 1992). Factors that potentially limit decomposition can be studied experimentally by adding them to decomposing litter in the field and measuring the effect on decomposition or decomposer organisms in comparison with an untreated control, as was done with water by Cornejo *et al.* (1994) and certain nutrients by Cuevas and Medina (1988).

Decomposition constants

Various functions can be fitted to decomposition and nutrient release data. The most common one is the single exponential model $W_t = W_0 e^{-kt}$, where W_0 and W_t are the mass or quantity of nutrient at the beginning of the experiment and after time t, respectively, and k is the decomposition or nutrient release constant. The time after which the material loses half its initial mass is obtained as $t_{1/2} = \ln 2/k$. As biomass usually consists of fractions that decompose and release their nutrients rapidly, and other more recalcitrant ones, double or higher exponential models have also been used that have coefficients for the proportion of material in each fraction and their respective decay constants. Linear models may well describe the initial phase of decomposition. These and other models and their respective advantages are discussed by Wieder and Lang (1982).

Decomposition standards

For the comparative assessment of decomposition processes at different sites, the inclusion of standard materials in decomposition experiments is useful. Proposed materials include birch (*Betula* spp.) sticks (Anderson and Ingram, 1993) and *Leucaena leucocephala* biomass (Budelman, 1988). Artificial substrates have also been used (Woods and Raison, 1982).

6.4 Measures of Resource Quality

The term resource quality is now prefered to the synonymous term substrate quality. It is a summary expression for the intrinsic characteristics

of an organic material that, together with the decomposer community and environmental factors, determine the speed of its decomposition and nutrient release (Swift *et al.*, 1979; Heal *et al.*, 1997). In a wider sense, characteristics that influence the fate of the carbon and nutrients derived from decomposing biomass, such as their incorporation into soil organic matter, may also be included in the term resource quality (Palm and Rowland, 1997). Both chemical and physical properties influence the quality of a resource, although the chemical ones seem to be generally more important and have been studied more thoroughly. Chemical and physical substrate characteristics can be separated experimentally by studying the decomposition of either the whole or ground materials (Agbim, 1987; Palm and Sanchez, 1991).

Methods for characterizing chemical resource quality have been reviewed by Palm and Rowland (1997), and the following recommendations are mostly taken from that paper. The chemical quality of an organic material is influenced both by its carbon constituents (carbon quality) and by its nutrient content and the chemical form of the nutrients (nutrient quality). Palm and Rowland (1997) proposed a minimum set of resource quality characteristics for decomposition studies, including lignin, soluble carbon, total nitrogen, total phosphorus, ash-free dry weight, and soluble phenolics if total nitrogen is greater than 1.8%. Measurement of the protein-binding capacity to assess reactive polyphenols may also be useful in some cases. For some characteristics, the choice of the analytical method is critical as different methods may produce different results, which leads to problems of comparability between studies. As for any laboratory analysis, the inclusion of reference materials that are exchanged with other laboratories is recommended.

Biomass samples are collected in more or less homogeneous units (e.g. old leaves, young leaves, branches), and the contribution of each unit to the total biomass is quantified. For the analysis of soluble compounds, the samples are dried at 35–40°C, otherwise a drying temperature of 60–80°C can be used (105°C for woody materials). All measurements should be referred to dry weight as obtained at this temperature. The dried samples are ground to pass a 1 mm sieve. The recommended procedure and methods for biomass analyses are given in Fig. 6.3. The alternative procedure in Fig. 6.4 still requires testing before routine application (Palm and Rowland, 1997).

Physical biomass characteristics that could influence decomposition include toughness, particle size and surface properties such as waxiness. In a litter layer, sclerophyllous leaves often have less contact with the soil than soft leaves, and this may influence their water content and decomposer activity. However, chemical and physical litter characteristics are not independent, and useful physical measures of litter quality have not yet been identified (Palm and Rowland, 1997).

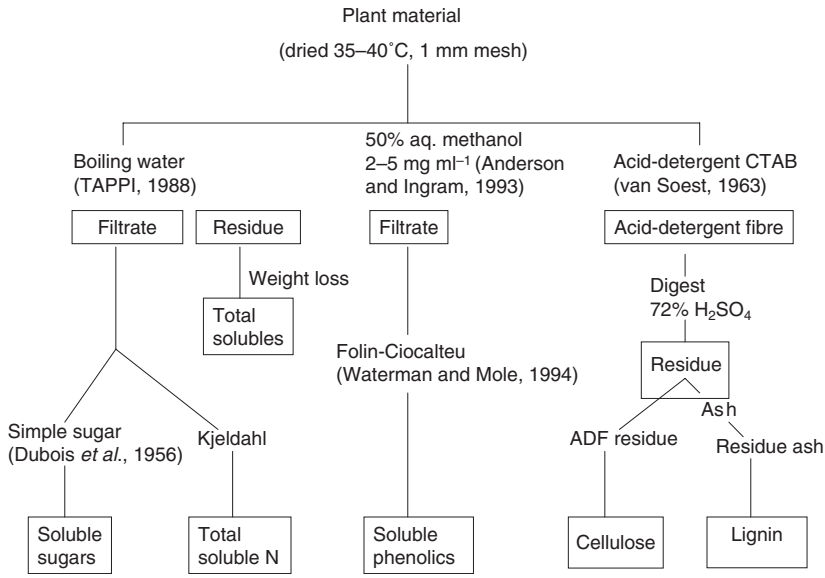


Fig. 6.3. Recommended procedures for analysis of carbon proximate fractions using three separate extractions (reproduced with permission from Palm and Rowland, 1997). CTAB, cetylmethyl ammonium bromide; ADF, acid-detergent fibre.

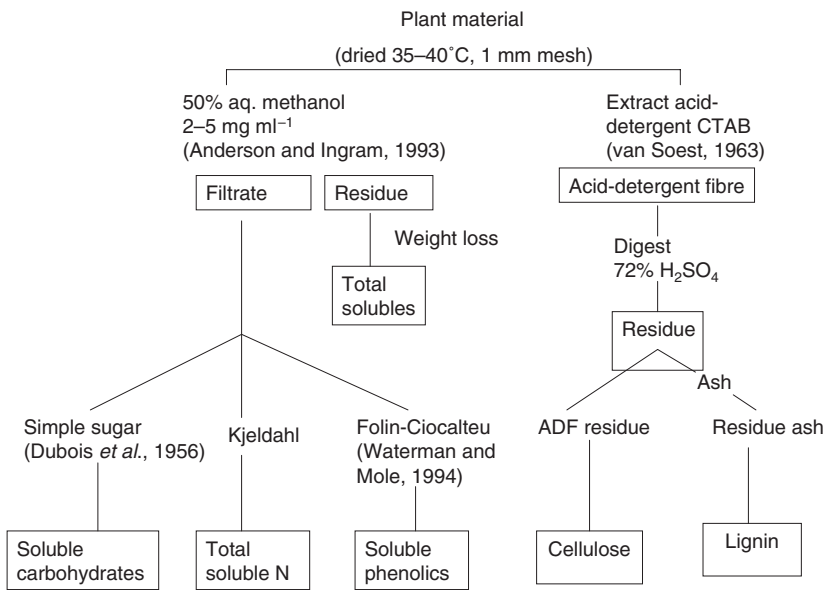


Fig. 6.4. Alternative procedure for analysis of carbon proximate fractions using two separate extractions (reproduced with permission from Palm and Rowland, 1997).

Chapter 7
Nutrient Leaching

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7.1 Synopsis

Nutrient leaching is the downward movement of dissolved nutrients in the soil profile with percolating water. Nutrients that are leached below the rooting zone of the vegetation are at least temporarily lost from the system, although they may be recycled if roots grow deeper. Leached nutrients may contribute to groundwater contamination in regions with intensive agriculture. Nitrate leaching is also a significant source of soil acidification. In humid climates, some nutrient leaching occurs even under natural vegetation, but agricultural activities can greatly increase leaching losses (Havlin *et al.*, 1999).

Soil and climatic factors that influence nutrient leaching

In general, water transport below the rooting zone requires that the soil water content exceeds field capacity and the water balance is positive, which means that water inputs with rainfall (and irrigation) exceed evapotranspiration. Therefore, nutrient losses through leaching are generally higher in humid than in dry climates (Havlin *et al.*, 1999). In certain soils, however, water can infiltrate into the subsoil through continuous vertical macropores when the bulk soil is dry. This is especially important in cracking clay soils (Vertisols) at the onset of the rainy season (Smaling and Bouma, 1992). Macropores are also created by faunal activity and root growth. They only conduct water under conditions of heavy

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rainfall or irrigation, under other conditions they are filled with air. Macropore or bypass flow may increase nutrient leaching following the surface application of fertilizers, because a solution with high nutrient concentration then infiltrates rapidly into the soil with little contact with the soil matrix. On the other hand, macropore flow may also protect nutrients present in smaller soil pores from being leached by rapidly channelling away surplus water (Cameron and Haynes, 1986; van Noordwijk *et al.*, 1991b).

Soils with high water infiltration rates and low nutrient retention capacity, such as sandy soils and well-structured ferrallitic soils with low-activity clays and low organic matter contents, are particularly conducive to nutrient leaching (von Uexküll, 1986). Some nutrients are easily leached from organic soils (see below). Subsoil acidity also tends to increase nutrient leaching by restricting the rooting depth of sensitive plants (see Section 5.6).

In the subsoil of many tropical soils the mobility of nitrate and other anions decreases because of increasingly positive net charge and, therefore, anion retention by soil minerals, and this increases the probability that these ions are eventually taken up by deep-rooting plants (see Box 8.1 on p. 171). It is, therefore, important to distinguish between nutrient leaching within the soil profile, from the topsoil into the subsoil, leading to temporary nutrient loss, and leaching beyond the rooting zone of deep-rooting plants, into the groundwater, leading to permanent nutrient loss.

Susceptibility of different nutrients to leaching

The leaching risk for a nutrient increases with its mobility in the soil. Among nutrient anions, nitrate is particularly easily leached because it shows negligible interaction with the negatively charged matrix of most topsoils and is, therefore, very mobile in the soil (see Section 5.2). Nitrification rates are variable in tropical soils, but can be sufficiently high to make nitrate the dominating form of mineral nitrogen even in acid soils (Robertson, 1989; Schroth *et al.*, 1999a). As a consequence, leaching may contribute significantly to negative nitrogen balances of agricultural systems (Smaling *et al.*, 1993). In seasonal climates, nitrate is also particularly exposed to leaching because a mineralization flush of organic nitrogen that causes release of large quantities of nitrate in the topsoil often occurs when dry soil is rewetted at the onset of the rainy season, at a time when crops have not yet been sown or are still small (Birch, 1960).

A mineralization flush at rewetting of dry soil has also been reported for sulphur (Havlin *et al.*, 1999). Sulphate is also readily leached from surface soils, the losses being highest in soils dominated by monovalent cations (potassium, sodium) and lowest in soils with high amounts of

aluminium (Havlin *et al.*, 1999) (see Section 5.4). Dissolved organic sulphur contributed between 18 and 86% of total dissolved sulphur at 2 m depth in an agroforestry system in central Amazonia (J. Lehmann, unpublished data).

In contrast to nitrate and sulphate, phosphate is immobile in most soils because of precipitation and adsorption to mineral surfaces, and leaching is therefore negligible, except in certain very sandy and organic soils (Wild, 1988) (see Section 5.3). Dissolved organic phosphorus forms are more mobile in soil than phosphate (Havlin *et al.*, 1999). Phosphorus may also be lost if surface soil particles are eroded in runoff (see Section 17.1).

The percolating soil solution that carries nutrients down the soil profile is necessarily electrically neutral; therefore, anions are leached together with equivalent amounts of cations. In most soils, the cations most likely to be leached are calcium and magnesium. In West African savanna soils, close relationships between the combined concentration of Ca and Mg and that of nitrate in the soil solution below the crop rooting zone have been reported, pointing to the role of fluxes of nitrate, and to a lesser extent chloride, as factors controlling calcium and magnesium leaching in these soils (Pieri, 1989). In sandy soils, considerable amounts of magnesium can be leached after applications of potassium chloride or potassium sulphate fertilizers (Havlin *et al.*, 1999). Potassium is usually leached in much smaller quantities than calcium and magnesium even when applied as fertilizer and was not related to nitrate fluxes in the aforementioned studies in West Africa (Pieri, 1989). However, significant potassium leaching may occur in sandy and organic soils and in high-rainfall areas (Malavolta, 1985; Havlin *et al.*, 1999). Among the micronutrients, manganese and boron are susceptible to leaching in certain soils (Havlin *et al.*, 1999).

Management practices that reduce nutrient leaching

A number of agricultural practices reduce nutrient losses through leaching by increasing the synchrony and synlocation of nutrient uptake by the vegetation with nutrient supply from soil, mineral fertilizers and organic materials (see Section 6.1). These include:

- early sowing of crops at the onset of the rainy season in savanna climates to make use of the mineralization flush of nitrogen upon rewetting of the soil (Myers *et al.*, 1994);
- rapid installation of a vegetation cover after forest or fallow clearing to avoid nutrient losses from bare soil (Webster and Wilson, 1980; von Uexküll, 1986);
- applying fertilizers (especially nitrogen) in several small applications during the cropping season rather than all at once; and

- placing fertilizer at the zone of maximum root activity of tree crops (IAEA, 1975; Havlin *et al*., 1999).

Leaching of nutrients from organic sources

Of particular relevance for agroforestry is the efficient management of nutrients in organic materials, including biomass and manure, for increased crop uptake and reduced leaching losses (see also Chapter 6). Nutrient release from organic sources is generally more difficult to predict than from mineral fertilizers and so developing practices to counteract leaching is particularly important. Nutrients are often released from organic sources at a time when there is little crop uptake and consequently more opportunity for leaching. Although leaching losses of nutrients from organic sources comparable to or even higher than from mineral sources have been reported (Havlin *et al.*, 1999), other results show lower leaching of nutrients from biomass than from mineral fertilizer. Snoeck (1995) applied ^{15}N-enriched urea or biomass from either *Leucaena leucocephala* or *Desmodium intortum* that was also enriched with ^{15}N to coffee plants on an Oxisol in Burundi and measured the distribution of the nitrogen in undecomposed biomass, coffee plants and soil after 1 year. Almost half of the urea nitrogen was lost from the system, presumably by leaching below 30 cm soil depth, but most of the nitrogen released from biomass was retained in the topsoil (Fig. 7.1). Lehmann *et al.* (1999c) found that sorghum (*Sorghum bicolor*) took up more nitrogen from labelled ammonium sulphate than from *Acacia saligna* leaves in a runoff agroforestry system in northern Kenya. Much of the fertilizer nitrogen that was not taken up by the crop was lost from the system by leaching or volatilization, whereas 99% of the biomass nitrogen was recovered in soil and crop at the end of the cropping season. This highlights the important point that labile nutrients are both more vulnerable to leaching and more readily taken up by crops, so in some circumstances farmers may tolerate higher leaching losses from mineral fertilizers because the short-term nutrient uptake by crops and crop yields may also be greater than from organic nutrient sources. Mechanisms responsible for lower leaching losses from biomass than from mineral sources include:

- slower nutrient release, which is especially important when relatively large quantities of nutrients are applied at a time, as in the latter study, and
- stimulation of microbial growth in the soil by organic nutrient sources, leading to temporary immobilization of nutrients in the microbial biomass.

Fig. 7.1. Distribution of ^{15}N-labelled nitrogen 1 year after application to coffee (*Coffea arabica*) plants as urea or biomass of *Leucaena leucocephala* or *Desmodium intortum* on an Oxisol in Burundi. Total quantities applied were 9.2 g per plant of urea-N vs. 34.4 g per plant of *Leucaena*-N (1225 g biomass with 2.81% N) and 20.5 g per plant of *Desmodium*-N (682 g biomass with 3.01% N). Note that absolute quantities of nitrogen taken up from urea and *Leucaena* biomass were similar (2.1 g per plant); differences in percentage uptake resulted from different quantities applied. Nitrogen uptake from *Desmodium* biomass was 1.2 g per plant (after Snoeck, 1995).

Effect of trees on nutrient leaching

One of the central hypotheses of agroforestry is that the continuous or intermittent presence of trees in land-use systems can increase the efficiency with which nutrients are retained in the soil–plant system and transformed into biomass and harvested products instead of being lost by leaching (Young, 1997). This hypothesis has been confirmed in a limited number of studies. Seyfried and Rao (1991) measured lower nutrient concentrations in the soil solution and calculated lower nutrient leaching in a multistrata agroforestry system with cocoa, banana and *Cordia alliodora* than in a maize monocrop in Costa Rica. Horst (1995) reported lower nitrate concentrations in the soil solution and consequently less leaching under hedgerow intercropping with *Leucaena leucocephala* and annual food crops than in the agricultural control treatments in southern Benin.

Lehmann *et al.* (1999a) measured lower nutrient leaching under an *Acacia saligna*–sorghum intercrop than under pure sorghum with runoff irrigation in northern Kenya.

Several mechanisms may contribute to reduced nutrient leaching under agroforestry compared with agricultural monocrops. Through increased litter, mulch and root production, agroforestry practices may contribute to increased soil organic matter levels and therefore increased cation exchange capacity and nutrient retention (see Chapter 4). Also, trees may create macropores with their roots or through the stimulation of macrofaunal activity (see Chapter 16), and this may help to channel surplus water through the soil with limited contact with nutrients in the soil matrix (bypass flow, see above and Chapters 10 and 11). These tree effects are desirable in both fallow rotations and simultaneous agroforestry systems. Furthermore, water uptake by trees may reduce water infiltration and, therefore, nutrient leaching. Lower soil water contents in agroforestry plots than in agricultural controls have often been reported (Malik and Sharma, 1990; Rao *et al.*, 1998). Trees may also reduce nutrient concentrations in the percolating soil solution through nutrient uptake (Horst, 1995). Reduction of nutrient leaching by trees through uptake of water or nutrients is only desirable in fallow systems and in tree–crop associations during periods when no crops are present, such as before sowing and after the harvest of annual crops. When both trees and crops are present in a field at the same time, water and nutrient uptake by trees may reduce nutrient leaching, but may also cause yield depressions of the crops through competition. These conflicting effects of trees in simultaneous agroforestry systems are apparently one reason why agroforestry associations such as hedgerow intercropping have often been successful in maintaining soil fertility at higher levels than agricultural controls, but have not improved crop yield (Rao *et al.*, 1998) (see also Chapter 5).

The safety-net hypothesis proposes that it is possible to achieve reduced nutrient leaching without increased root competition between trees and crops (van Noordwijk *et al.*, 1996). The hypothetical safety net for leached nutrients is formed by trees that possess few superficial roots, but whose deep roots spread laterally below the rooting zone of associated, shallow-rooting crops. Here, in the subsoil, they intercept nutrients and water, thereby reducing nutrient leaching without being associated with too much competition with the crops in the topsoil. The safety-net concept is an idealization that guides the search for tree species that exhibit a high degree of niche differentiation with crops in that they have relatively uncompetitive root systems in surface soil, but are sufficiently deep-rooting to acquire relevant amounts of subsoil resources. Utilization of subsoil nitrogen by a deep-rooting, uncompetitive tree species, *Peltophorum dasyrrhachis*, in association with groundnut on an Ultisol in Sumatra has

been demonstrated, where the tree obtained more than 40% of its nitrogen from below the crop rooting zone (Rowe *et al.*, 1999). Further support for the hypothesis comes from the observation of stratified root systems of associated plant species in different natural and artificial ecosystems, which develop either because the vertical root distribution of associated species responds differently to soil and climatic factors, or because the root system of one species avoids competition from the other species through increased growth in the subsoil (Schroth, 1999). However, a clear experimental demonstration of a safety-net effect in terms of lower nutrient leaching in agroforestry than in agricultural systems that is not offset by competition for water and nutrients in the topsoil is still lacking.

Another way in which agroforestry (or intercropping) techniques can reduce nutrient leaching is by optimizing the spatial patterns of nutrient use in tree crop plantations. If tree crops are planted at final spacing, they often take several years to fully occupy the soil with their root systems, and during this time nutrients released from the soil, a cover crop or decomposing residues from the previous vegetation may be leached in the spaces between the tree crop plants unless these are occupied by suitable intercrops, shade trees or spontaneous vegetation. Nitrate leaching in the spaces between 5-year-old widely spaced tree crops (peach palm – *Bactris gasipaes*, cupuaçu – *Theobroma grandiflorum*, Brazil nut – *Bertholletia excelsa* and annatto – *Bixa orellana*) has been reported from an Amazonian Oxisol, indicating the presence of surplus nutrients and water that could be used for additional crop production (Schroth *et al.*, 1999a). On a similar soil, the nitrate distribution in the soil under a 15-year-old oil palm plantation indicated that interplanting with shade-tolerant crops may still have been viable at this age (see Fig. 8.1 on p. 170). Similarly, in mature plantations of coconut (*Cocos nucifera*) significant quantities of light, water and nutrients may not be captured by the tree crops and so can be utilized by intercrops without reducing tree crop yield (Mialet-Serra *et al.*, 2001). The potential of tree crop-based agroforestry systems to reduce nutrient leaching has been discussed in more detail by Schroth *et al.* (2001b).

In contrast to simultaneous tree–crop associations, fallow systems rely on the rapid development of deep roots of the fallow trees to intercept nutrients in the subsoil which were leached during the previous cropping phase (see Chapter 8). Modelling results suggest that, under high-leaching conditions and for very mobile nutrients such as nitrate, fallows may not be effective in recycling leached nutrients; instead, the permanent presence of tree roots as in simultaneous systems would be necessary to intercept these nutrients before they are leached too deep into the subsoil (van Noordwijk, 1989). As discussed in Box 8.1 on p. 171, anion retention in the subsoil of many tropical soils increases the potential for intermittent fallows with deep-rooting trees to recycle leached nitrate, suggesting that leaching losses could be reduced at a lower competition cost than with

permanent tree–crop associations. Experimental comparisons of simultaneous and sequential agroforestry systems under different pedoclimatic conditions with respect to nutrient cycling and overall productivity would be required to confirm this.

7.2 Methods for Soil Solution Composition

The soil solution comprises the soil water and the inorganic and organic substances that it contains. Nutrient leaching can be determined from the quantity and composition of the soil solution that percolates to depths greater than the rooting depth of the plant species present. Depending on the respective research question, the collection of soil solution may be necessary either below the rooting zone of an annual crop, which could be anything from a few decimetres to a few metres deep, or below the rooting zone of a tree, which may be many metres deep. For technical reasons, solution sampling below the deepest roots of agroforestry trees is often not feasible. However, measurements of the soil solution composition and nutrient leaching at different depths under crops and trees may still provide valuable information on the ability of the plant species to capture nutrients from the percolating solution in the subsoil.

There are three approaches to the measurement of nutrient leaching through the collection and analysis of soil solution (Fig. 7.2).

- Solution samples are collected and leaching is estimated from the concentration of dissolved nutrients and the movement of the solution in the soil, which is measured separately. Suction cups are the most common tools for soil solution sampling. A more recent development, the tensionic sampler, allows soil solution measurements without application of suction due to diffusion of solutes through a porous cup

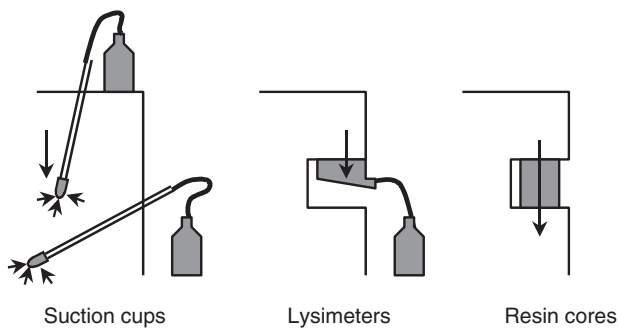


Fig. 7.2. Schematic depiction of the installation of suction cups, lysimeters and resin cores. The arrows indicate the direction of solution movement through the soil and into the collectors.

into the sampling device. As no suction is applied, the same device can be used for tensiometer readings (Moutonnet *et al*., 1993). Alternative methods include the extraction of solution from field-moist soil by centrifugation or displacement with different immiscible liquids, but these techniques require destructive soil sampling for every solution measurement and are thus not suitable for determining cumulative nutrient losses during longer time intervals. Examples from agroforestry experimentation using suction cups and a water balance for leaching measurements include Seyfried and Rao (1991), Kühne (1993) and Lehmann *et al*. (1999a).

- The percolating solution is collected in lysimeters with a defined collection area, so that the downward flux of the solution can be determined from the collected sample volume, which is also used for nutrient analysis. A principal difference exists between tension lysimeters (including suction plates) and free-draining lysimeters. An agroforestry application of the latter technique is described by Santana and Cabala-Rosand (1982).
- The percolating solution passes through a sampler with a defined area, in which nutrients are retained on an ion-exchange resin; in this method the percolating water volume does not need to be measured. This inexpensive and simple technique is still under development. Agroforestry applications include Hagedorn *et al*. (1997) and Lehmann *et al*. (1999b).

Suction cups

Suction cups are fine-porous filters that are closed at one end and mounted on a rigid or flexible tube at the other end. The dimensions of the cups vary in general between 3 and 60 cm in length and between 0.5 and 10 cm in diameter. The soil solution is sucked through the cups and into a collection bottle by applying a vacuum, either temporarily or continuously. The vacuum applied to suction cups has to be adjusted in order to sample only the most mobile water, although it needs to be strong enough to gather enough soil solution for analysis. This can be a problem in very clayey soils. Battery-powered pumps can continuously keep the vacuum at the desired level. Where the soil water content shows pronounced fluctuations during the sampling, periodic adjustments of the applied vacuum may be necessary. Self-regulating devices are now available, which measure the soil water suction and automatically adjust the applied vacuum.

Suction cups are well suited for studies of short-term fluctuations and small-scale variability of soil solution chemistry, because the solution can be collected at short time intervals, and even the installation of a large

number of cups at depths of several metres causes little disturbance in a plot. Shortcomings of suction cups are that the mobile soil solution is not adequately sampled in most cases and that the collected solution cannot be related to a defined soil volume or infiltration area (Grossmann and Udluft, 1991). Preferential water flow through macropores may constitute the majority of the percolating solution, but its infiltration is often too rapid to be sampled with suction cups. Failure to sample the initial mobile soil water after rewetting may result in large errors in estimates of leaching, as this water may be enriched with nutrients (Lord and Shepherd, 1993). The suction cup technique may yield reliable data in sandy, unstructured soils (Webster *et al.*, 1993), whereas clayey and well-structured soils may pose considerable difficulties.

Various materials are used for the cups, which differ in their purpose and applicability. Cups made from P80 ceramic material with a pore diameter of about 1 μm are frequently used because of their low price. In these cups, however, significant amounts of phosphate and dissolved organic matter may be retained in the material. If organic compounds are of interest, therefore, the suction cups have to be conditioned for a long time in the soil being studied. More inert materials for phosphorus sampling include Teflon, glass, polytetrafluoroethene (PTFE)/quartz or cellulose acetate fibres (Dorrance *et al.*, 1991; Beier and Hansen, 1992). Pretreatment of the cups with dilute hydrochloric acid, followed by distilled water, before installation is generally necessary (Angle *et al.*, 1991; Beier and Hansen, 1992). This also eliminates problems with aluminium desorption from P80 cups. The glues connecting the cups with shafts may leak organic substances but contamination problems can be avoided by reducing the contact zone of solution and glue or by using glue-free suction cups. The installation should be done well in advance of the intended measurements to allow for chemical equilibration of the cups with the surrounding soil and to let the soil settle after the installation (Lord and Shepherd, 1993; Webster *et al.*, 1993).

To avoid microbial transformations, the collected solution should be frequently removed from the collection bottles and should be either analysed immediately or deep-frozen (Angle *et al.*, 1991). In practice, the solution often remains in the collection bottles in the field (dark bottles are preferred to prevent the growth of algae) or in the suction cups for a few hours or days before it is collected. Chemicals can be added to the bottles to inhibit microbial activity, such as chloroform or various acids. These additions, however, may interfere with the intended measurements of pH, certain colorimetric reactions, and dissolved organic matter. Alternatively, the solution can be collected without preservation and be digested prior to the analysis of elements that could be affected by microbial growth (especially nitrogen and phosphorus). In this case, only total fluxes of these nutrients are determined without specifying the chemical compounds.

Collection of the solution from several samplers in the same bottle or pooling of collected solution prior to analysis are common practices to obtain more representative samples without increasing the number of analyses. However, pooling may increase the risk of losing the whole sample if one cup produces contaminated solution. It is thus advisable to analyse samples from all individual cups at the beginning of a measurement programme.

When installing the cups, care has to be taken to avoid preferential water flow along the shafts by sealing with clay or rubber discs. At shallow depths, the cups are often installed at an angle to the soil surface. At greater depths, they may be installed horizontally from a soil pit. After long-term use, the connectors and tubings of the suction cups may show signs of ageing and not hold the vacuum any more. Termites and ants may destroy plastic tubes, and polyethylene materials are also susceptible to light damage. In soils rich in dispersible clay, the cup pores can become clogged after prolonged use, in which case the cups have to be replaced.

Lysimeters

Lysimeters as defined here are horizontally installed trays that capture percolating soil water. Discussions of the technique can be found in Dorrance *et al.* (1991), Barbee and Brown (1986) and Russell and Ewel (1985). Free-draining lysimeters are open at the top and rely on gravity for collecting the percolating solution (Jordan, 1968). In contrast, tension lysimeters (or suction plates) consist of porous materials similar to those used in suction cups with a sealed bottom, to which a vacuum is applied. This vacuum should ideally resemble the matrix potential of the underlying soil so that the water percolation is not influenced by the sampler. Unlike suction cups, lysimeters can also sample macropore flow.

Free-draining lysimeters have been found to yield more soil solution than suction cups in clayey soils (Barbee and Brown, 1986). However, they will still underestimate percolation because the soil water has to overcome the soil matrix potential to enter the collector, and in finely textured soil with weak soil structure no water at all may be collected. The sampling technique may also influence the solution chemistry. For example, free-draining lysimeters were found to yield solution from a wetting front later and with higher calcium and potassium concentrations than suction cups, which collected a solution with higher nitrogen and magnesium concentrations (Marques *et al.*, 1996). In an unstructured soil, similar results for both methods have been obtained (Webster *et al.*, 1993).

In experiments with annual crops or before tree planting, lysimeters can be installed from the soil surface below the plough layer. For greater installation depths and with an established tree root system, the installation

has to be carried out from the side using a soil pit. Special care has to be taken to ensure a good contact between the lysimeter and the overlying soil (Barbee and Brown, 1986). The same rules as for suction cups apply to selection and pretreatment of the porous material and preservation of the collected solution.

Resin cores

Resin cores are cylinders filled with ion-exchange resin and installed in the soil in a way that allows water percolation (Schnabel, 1983). Nutrients in the percolating solution are adsorbed to the resin and can be extracted after the core has been removed. Similarly to lysimeters, resin cores have a defined surface area, so that a time integral of nutrient leaching per unit area is obtained. Depending on the leaching rate and the construction of the cores, they can be left in the soil for periods as long as 6–12 months. Disadvantages of this technique are that collection of the resin is destructive and that short-term leaching events are not detected due to the integrative nature of the measurement.

The cores usually have a length and diameter of 5–20 cm and can be built from PVC tubing. The water flux through the cores relative to the surrounding soil and hence the validity of the results will strongly depend on the similarity between resin cores and soil with respect to water permeability at different water contents. To increase the similarity of hydraulic properties between cores and soil, the resin should be mixed with acid-washed sand or soil (Hagedorn *et al.*, 1997). Even with such precautions, it may be difficult to determine absolute leaching losses in finely textured soils. The collection efficiency of resin cores can be checked by tracer experiments using chloride.

Commercially available exchange resins used for water purification can be used in the cores since they show low blank values and high recovery rates for nitrate, ammonium, phosphate and basic cations. However, the resin properties should be checked in preliminary adsorption experiments. Dissolved organic matter and organic nutrients are often difficult to determine with this method, because inexpensive resins may bleed organic compounds and the necessary quantities of analytical resins may not be affordable (Lehmann *et al.*, 2001d).

At the end of the measurement period, the resin cores are recovered and the resin is extracted with KCl or $CaCl_2$ solution, depending on the nutrients to be analysed. The cores are cut and different resin layers extracted separately to make sure that the nutrient load of the percolated solution did not exceed the exchange capacity of the resin. The lowest layer may contain a large portion of nutrients derived from capillary rise and should be excluded from the calculation of nutrient leaching.

Resin cores should not be confused with resin bags, which are placed in the soil to obtain a time-integral of the availability of certain nutrients without an intention to measure nutrient leaching (Binkley, 1984).

7.3 Tracer Methods for Nutrient Leaching

Several different approaches exist for employing tracers to measure nutrient leaching. Their suitability depends on experimental objectives. First, it depends on the source of the nutrients whose leaching is being assessed. These may be derived from precipitation, nutrient mineralization in the topsoil, mineral fertilizer or organic materials such as prunings or manure. The source of the nutrient will determine the chemical form in which the tracer is best applied, for example, as a component of a mulch material or as a mineral fertilizer. Secondly, the choice of method will be influenced by the question as to which of the various processes that determine nutrient leaching need to be considered in the experiment. For example, different methods would be applicable if there were only interest in water percolation as influenced by the availability of water and the infiltration rate of the soil than if it were also necessary to understand nutrient uptake by plants.

There are two different approaches to the measurement of nutrient leaching with tracers:

- measuring the content of the tracer in the soil to a certain depth, comparing the recovered quantity with the amount that was applied (or measured in the same soil volume at an earlier date) and considering the difference as leached (for nitrate profiles see Box 8.1 on p. 171); and
- measuring the amount of tracer that passes through a certain soil depth (such as the maximum rooting depth of a crop) with the techniques described in Section 7.2.

A large number of different tracers exist, which can be classified as either:

- radioactive isotopes (e.g. ^{32}P, ^{33}P, ^{35}S);
- stable isotopes (e.g. ^{15}N, ^{34}S); or
- other tracers (e.g. strontium, lithium, rubidium, bromide or chloride).

Radioisotopes and stable isotopes allow use of the same nutrient for which the leaching is to be determined without significantly changing its concentration in the soil. Non-isotopic tracers can simulate the behaviour of certain chemically related nutrients in soil, for example, chloride and bromide can be used for nitrate, strontium for calcium, and rubidium and lithium for potassium (see also Section 8.2). However, higher amounts of

the tracers may have to be used to be detectable because of the low sensitivity of the analyses or sometimes the high amount of the substance already present in the environment. If nutrient leaching from organic sources or the effect of nutrient uptake by plants on nutrient leaching is to be studied, only radioactive or stable isotopes of nutrient elements can be used, because only these possess similar properties during decomposition and microbial transformation to the nutrient of interest. However, radioisotopes will in many cases be considered too dangerous for a field assessment of nutrient leaching, leaving stable isotopes as the only option.

Tracers can be applied to the soil in mineral form or in organic materials such as mulch, litter, compost or manure. In the latter case, the substrate has to be labelled beforehand. This can be done by supplying the tracer to a plant whose litter or biomass is to be used in the experiment and collecting the respective materials after some weeks. The application method depends on the objectives. Labelled fertilizer will usually be applied in the same manner as unlabelled fertilizer. If the emphasis of the study is on the assessment of the nutrient transport in the soil rather than the release from a certain nutrient source, the tracer is best applied in solution, which can be applied more evenly than solid materials such as fertilizer. A simple hand-sprayer or electric pump can be used to spray a known amount of the dissolved tracer on a defined area. The amount to be applied will depend on the amount already present in the soil and the duration of the experiment. For example, ^{15}N can be applied at a dose of 1 g m^{-2} to be detectable even after several months in cropping systems with trees.

If the purpose of the study is to examine nutrient leaching after fertilization, large amounts of nutrients are usually applied to the soil. This may cause pool substitution of the tracer with native soil nutrients, as reviewed for nitrogen isotopes by Jenkinson *et al.* (1985). As a result, more unlabelled soil nitrogen may be leached with fertilization using a labelled nitrogen source than without, and total nitrogen leaching as affected by fertilization may, therefore, be underestimated. Only by measuring total nutrient losses together with tracer losses can the extent of the added nitrogen effect be determined and conclusions drawn about the leaching of applied and native soil nitrogen.

Non-isotopic tracers are useful as long as nutrient uptake by plants does not need to be considered. For example, nutrient leaching at the onset of the rains before crop planting has been studied with bromide as a tracer in an East African Vertisol (Sticksel *et al.*, 1996). Walker *et al.* (1991) used the chloride content of wet deposition for measuring groundwater recharge as affected by land-use change in semiarid Australia. Also, hydrogen isotopes can be used to monitor water movements in soil (Münnich, 1983). However, in the presence of vegetation, these tracers

would only reflect the effect of plant water uptake on leaching, but not the effect of actual nutrient uptake by plants from the percolating soil solution. Such data can be combined with measurements of nutrient concentrations in the soil solution (see Section 7.2) to determine nutrient leaching.

The tracers can be retrieved for the analysis by collecting the soil solution (see Section 7.2), extracting the soil with a salt solution or a dilute acid (see section for the respective element in Chapter 5) or, in the case of nitrogen, be analysed directly by dry combustion of the soil (Barrie *et al.*, 1995).

Nitrogen occurs in the soil solution mainly as nitrate, ammonium and dissolved organic nitrogen (see Section 5.2). These nitrogen forms differ in their behaviour with respect to:

- the soil matrix in terms of their adsorption and diffusion;
- soil microorganisms in terms of nitrification, denitrification and mineralization; and
- plant uptake.

The analysis of ^{15}N is mostly done after the combustion of the entire soil sample. If separate analyses of the different nitrogen forms are of interest for the study, the sample has to be fractionated before the ^{15}N analysis. The separate analyses of nitrate-^{15}N and ammonium-^{15}N, for example, in soil solution samples or KCl extracts, is possible after steam distillation (Bremner and Edwards, 1965; Buresh *et al.*, 1982) or diffusion (Brooks *et al.*, 1989). The distillates are freeze-dried or dried by diffusion. The dried samples can be directly analysed by isotope mass spectrometry. Distillation is more laborious and expensive than diffusion and subject to a higher analytical error since cross-contamination can easily occur. Several ways of reducing this problem have been discussed by Mulvaney (1986) and Mulvaney *et al.* (1994).

In certain ecosystems, a large proportion of the total nitrogen in solution can be in an organic form, and it may then be interesting to consider organic nitrogen forms in leaching studies. The amount of ^{15}N in organic form can be obtained by subtracting the inorganic ^{15}N (ammonium + nitrate ^{15}N) from the total ^{15}N in the soil solution. Further fractionation into hydrophilic and hydrophobic organic nitrogen is also possible (Qualls and Haines, 1991).

7.4 Dyes as Tracers for Preferential Flow Paths

Water infiltration and nutrient movements in soil are strongly influenced by macropores (see Section 7.1). The macroporosity of soil can be influenced by tree roots and burrowing soil animals, whose activity may be favoured by the presence of tree litter (see Section 10.1). Laboratory

and field procedures for measuring soil porosity and water infiltration are discussed in Sections 10.4 and 11.4. Dyes are useful for staining flow pathways in the soil, so that these can be analysed in relation to other characteristics, such as the distribution of roots, faunal structures (van Noordwijk *et al.*, 1993b) or soil microbial properties (Bundt *et al.*, 2001). A disadvantage is that the infiltration of dyes is often limited to a few decimetres from the depth of application. Dyes that have been successfully used include rhodamine-B (Douglas, 1986), acid-red 1 (Ghodrati and Jury, 1990), methylene blue (van Noordwijk *et al.*, 1991b) and brilliant blue (Bundt *et al.*, 2001). Van Ommen *et al.* (1988, 1989) detected preferential flow paths in soil to 70 cm depth by infiltrating a solution of iodine, which does not interact much with the matrix of most soils. The iodine is transformed into a coloured complex after treating the exposed soil with starch and Cl_2. Dyes can be applied in the field by spraying on the soil surface, or through metal rings or bore holes as in infiltration measurements (see Section 11.4). Flow paths are evaluated in vertical trenches and/or by successively removing horizontal soil layers (Ghodrati and Jury, 1990).

Chapter 8
Nutrient Capture

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8.1 Synopsis

An ecologically important difference between trees and annual crops is that, due to their longer lifetime, trees can form larger root systems and can thus take up nutrients and water from a greater volume of soil. Where compact soil layers restrict the growth of annuals but less so of trees, this gives an additional advantage to the trees. Depending on species and environment, important differences between trees and annual crops may exist both in the vertical and in the horizontal extension of the root systems. Where tree roots reach deeper than the roots of annual crops, they may be able to take up nutrients and water from the subsoil which are not accessible to the crops, and this may improve tree growth without leading to a proportionate increase in competition with associated crops (see complementarity in Box 5.2 on p. 101). The same occurs in environments with patchy nutrient distribution in the horizontal plane, where far-reaching lateral roots may enable trees to tap nutrients at a distance which crops cannot acquire. This may include nutrient-rich patches in depressions that receive run-on water and fertile sediments along watercourses.

There are different ways in which nutrients that have been taken up by trees from the deeper subsoil or fertile soil patches can become available to crops. When the trees are cut, for example at the end of a fallow, nutrients stored in their biomass may become available to crops provided that the tree material decomposes on site. (Where fallow vegetation is burnt, some of the nutrients will be recycled but a proportion, particularly

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of the nitrogen, will be lost; see Chapter 9.) Nutrients are also released into the soil from living trees through pruning, litterfall, dying roots or crown leaching and may thus become available to associated crops. However, the trees compensate for these nutrient losses through nutrient uptake from the soil. Therefore, crops are most likely to profit from increased nutrient capture by associated trees when they are efficient competitors for these nutrients once they have been released into the soil. Competitive crops could force associated trees to take up a substantial part of their nutrients from deep or laterally distant soil and could then scavenge a share of these nutrients when they are released into the soil from decomposing tree litter or prunings (Schroth *et al.*, 2001b). Nutrients may also be recycled from trees when animals feed on tree fruits and foliage and then drop their excreta close to the trees. Where animals congregate under trees or birds perch on branches, they may concentrate nutrients close to the trees because of enhanced deposition of material derived from foraging in a wider area in the vicinity of the tree. This latter effect would not be called nutrient capture by trees, although the effects are similar.

Nutrient capture from the subsoil: 'nutrient pumping'

Nutrient capture by trees from the subsoil can include nutrients released by weathering of primary minerals and also nutrients leached from the topsoil that are then recycled by the trees. Capture of newly weathered nutrients is restricted to relatively young soils where weatherable minerals still occur within the reach of tree root systems, including colluvial or alluvial soils with irregular nutrient distribution with soil depth.

Despite the prominence of nutrient pumping by trees as a theoretical concept in agroforestry, the actual importance of the process in different ecosystems and agroecological situations is not very well documented. Studies on tree crops in seasonally dry tropical climates have shown that nutrient uptake from the subsoil increases when the surface soil dries out (Comerford *et al.*, 1984), and the process could, therefore, be expected to be important in savanna areas. However, in West African savannas, Kessler and Breman (1991) assume that nutrient pumping by trees is of limited importance, because most trees present in the landscape are too shallow rooted (despite the occurrence of some very deep-rooted species), and many soils are either too shallow or too dry and nutrient-poor at depth to make nutrient pumping a useful strategy. Other authors, in contrast, have stressed the importance of deep-rooting trees with wide distributions in the region. *Acacia senegal*, *Acacia tortilis*, *Faidherbia albida* and *Azadirachta indica* were all found to root down to the water table at from 16 m to 35 m depth in sandy soils in Senegal (Leakey *et al.*, 1999) and there is evidence

of large nitrate reserves at depth in groundwaters in arid zones (Edmunds and Gaye, 1997). Use of water from the deeper subsoil (>2 m depth) during the dry season has been demonstrated for some woody species in the Brazilian *cerrado* savanna (Jackson *et al.*, 1999). In a savanna in Belize, on the other hand, pines (*Pinus* spp.) had tap roots, but other trees were shallow-rooted, and nutrient enrichment under their canopy could not be explained by nutrient recycling from the deeper subsoil (Kellman, 1979). Not surprisingly, where exotic tree species, selected for fast above-ground growth, have been grown with crops in seasonally dry environments, the trees have generally been shallow-rooted and competitive with associated crops (Sinclair, 1996). Clearly, nutrient capture at depth in natural and managed semiarid environments appears to be very site and species specific and it makes sense to look for complementarity in natural associations between tree and herbaceous species on particular site types to find candidate species for use in agroforestry.

The recycling of leached nutrients from the subsoil by deep tree roots could be a relevant process in humid and subhumid regions, where nitrate derived from mineral fertilizer or organic sources is rapidly leached out of the topsoil together with nutrient cations such as calcium and magnesium during the rainy season (see Section 7.1). Soils with a high percentage of kaolinite and oxides of iron and aluminium, such as Oxisols and Ultisols, or soils of volcanic origin that are rich in allophane can have a significant anion exchange capacity that enables them to retain nitrate by sorption to the mineral phase. Anion exchange capacity (and nitrate sorption) increases with decreasing pH and decreasing organic matter content of the soil. They are, therefore, usually negligible in the topsoil and are most pronounced in acidic subsoil horizons. Nitrate sorption isotherms, describing the increase of nitrate sorption with increasing concentration of nitrate in solution, for different depths of Amazonian and Kenyan Oxisols can be found in Cahn *et al.* (1992) and Hartemink *et al.* (1996), respectively.

By slowing the downward movement of nitrate, anion sorption increases the probability that part of this nitrate is taken up by deep roots and returned to the topsoil through litterfall, prunings or leaching from the tree crowns. Nitrate accumulations have been reported from different tropical regions under annual crops (Cahn *et al.*, 1993; Weier and Macrae, 1993; Hartemink *et al.*, 1996; Jama *et al.*, 1998) and also under a coffee plantation in Kenya (Michori, 1993, cited in Buresh and Tian, 1998), multistrata agroforestry and tree crop monocultures in central Amazonia (Schroth *et al.*, 1999a, 2000a) and a runoff agroforestry system in semiarid northern Kenya (Lehmann *et al.*, 1999a) (see also Box 8.1). Trees in humid tropical regions have often been seen as shallow-rooted, but it is now established that their roots can reach many metres deep (Nepstad *et al.*, 1994; Canadell *et al.*, 1996), provided that compact, acid

or otherwise unfavourable subsoil conditions do not impede deep root development.

Studies in western Kenya showed that planted fallows of fast-growing trees (*Calliandra calothyrsus*, *Sesbania sesban*) produced root length densities of >0.1 cm cm^{-3} to below 1.5 m soil depth and reduced soil nitrate at 0–2 m depth by 150–200 kg ha^{-1} within 11 months after their establishment (Jama *et al.*, 1998). They thereby recycled considerable amounts of subsoil nutrients, which could be made available to subsequent crops (or crops on neighbouring fields) through application of the tree biomass, either directly or after use as animal fodder. Factors that influenced these very promising results were certainly the fast growth of the trees, the high planting density of 1 m × 1 m, and the fact that the soils presented no obstacles for tree root development (Jama *et al.*, 1998). They thus illustrate the considerable potential of planted fallows, fodder banks and fuelwood plantations for nutrient recycling, but further research is necessary on the efficiency of trees in subsoil nutrient capture under other environmental and management conditions. Where tree spacing is wide, as in many tree–crop associations, tree root growth in the subsoil may be less than in closely spaced fallows because there is less intraspecific root competition in the topsoil. Also, the efficiency of widely spaced trees in nutrient recycling from the subsoil may be limited to a certain area close to the trees to which the deep tree roots have access (Fig. 8.1). Therefore, the evaluation of spatial patterns of root distribution and activity must be part of studies of nutrient leaching and recycling. Many tropical soils also

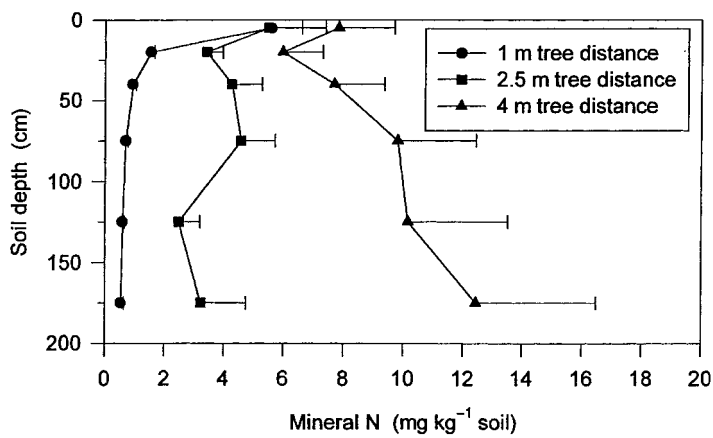


Fig. 8.1. Distribution of mineral nitrogen (nitrate-N + ammonium-N, means and s.e.) in the soil of an oil palm (*Elaeis guineensis*) plantation in central Amazonia at different tree distances, reflecting the restricted lateral root extension of the palms at depth (reproduced with permission from Schroth *et al.*, 2000a). For corresponding root distribution data see Fig. 12.1.

present impediments to tree root growth in the subsoil and hence nutrient pumping, such as hardened, very nutrient-poor or very acidic horizons (Kessler and Breman, 1991). Furthermore, there are indications that regular and frequent shoot pruning, which is a common management practice for trees in agroforestry, can reduce the rooting depth of trees, either through changes in root system architecture (van Noordwijk *et al.*, 1991a), or through a general reduction in root biomass. Such effects of shoot pruning have been found to be tree species specific (Jones *et al.*, 1998). All these factors need to be taken into consideration in future research on subsoil nutrient capture in agroforestry.

Box 8.1. Nitrate profiles as indicators of nitrogen leaching and recycling.

Leaching of nitrate and its recycling from the subsoil through deep-rooting plants are particularly relevant processes in agroforestry because nitrogen availability often limits crop yields, and nitrate leaching contributes to cation loss and soil acidification and may be a source of groundwater contamination (see Sections 5.2 and 7.1). The easiest way of obtaining information about nitrate leaching and recycling is usually by collecting soil samples from different depths and extracting the nitrate as described in Section 5.2. Shepherd *et al.* (2000) compared the nitrate

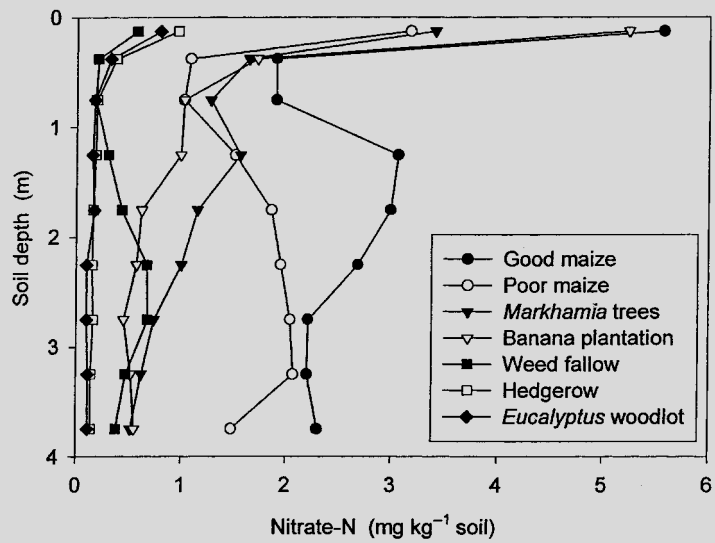


Fig. 8.2. The distribution of nitrate-N under seven land-use types and plant species on smallholder farms in Kenya illustrates the tighter nutrient cycles in systems with trees and perennial crops compared with annual maize (reproduced with permission from Shepherd *et al.*, 2000).

Continued

Box 8.1. *Continued.*
distribution to 4 m depth under different land-use types, including annual and perennial crops, trees and spontaneous fallow, in smallholder production systems in Kenya and found the largest accumulations of nitrate in the subsoil under maize (Fig. 8.2). Although such information does not allow quantification of nitrate losses from the different systems, the results demonstrate the advantage of integrating trees and perennial crops in annual cropping systems for closer nutrient cycles.

Using a similar approach, Jama *et al.* (1998) compared five tree species with respect to their ability to use nitrate that had accumulated in the subsoil during previous maize crops in Kenya as a criterion for their value in improved fallows. Nitrate profiles under different tree crops and a cover crop in a multistrata agroforestry system in Amazonia showed that the leguminous cover crop contributed more to nitrate leaching than three out of four tree crops in the system (Schroth *et al.*, 1999a). Information on such small-scale patterns of nitrate distribution can help to optimize the design and management of complex cropping systems by showing where in a system nutrients are used inefficiently and where additional plants could be added or fertilizer rates reduced. Ideally, such information would be combined with quantitative nutrient balances for the whole system, as obtained, for example, in catchment studies.

Nitrate profiles can also provide useful information about the soil volume from which crops or trees take up nutrients. The extension and shape of the rooting zone of trees, especially in the subsoil, is important information for evaluating their potential role in nutrient cycling in agroforestry systems. For example, for functioning as a safety net for nutrients leached from soil in which associated annual crops are growing (see Section 7.1), tree root systems need a sufficient lateral extension in the subsoil under the crop rooting zone (van Noordwijk *et al.*, 1996). Information on tree root distribution in the subsoil is difficult to collect through root studies and is, therefore, hardly ever available. However, by studying the spatial patterns of nitrate distribution in the soil, information about the lateral root extension at depth and the spatial patterns of nitrate leaching can be inferred with relatively little effort, as shown in Fig. 8.1. A similar approach can be used to analyse the influence of scattered trees, boundary plantings and hedgerows on subsoil nutrients in agroforestry associations (Mekonnen *et al.*, 1999).

Repeated measurements of the nitrate distribution in the soil in the same area, for example at the beginning and the end of a cropping season or a fallow period, provide information on temporal changes of nitrate concentrations at different soil depths, and these may be related to nitrate leaching and uptake. Hartemink *et al.* (1996, 2000) showed that nitrate in the subsoil (50–200 cm) of two Kenyan soils decreased under *Sesbania sesban* and weed fallows, but not under maize and concluded that subsoil nutrients and water were used more efficiently by the fallows than by the crops.

Nitrate profiles do not usually allow measurement of nitrate fluxes in a strictly quantitative sense, because several processes which simultaneously influence nitrate concentrations at a given soil depth cannot easily be distinguished. Nitrate accumulations in the subsoil may be the result either of leaching of nitrate from the topsoil, or of *in situ* mineralization of nitrogen, or of a combination of both processes. Weier and MacRae (1993) measured the nitrogen mineralization rates

at different depths in an Australian soil where nitrate was known to accumulate in the subsoil below shallow-rooted crops. They concluded that most of the subsoil nitrate had been leached from the topsoil, although *in situ* mineralization of nitrogen could make a contribution to the nitrate accumulation in the subsoil under black gram (*Vigna mungo*). Similar information from other sites would be helpful in the interpretation of nitrate profiles.

Decreases in subsoil nitrate content may also be caused by several processes, which may occur simultaneously. First, nitrate may be taken up by the vegetation, such as by deep-rooting trees. This requires that roots extend to the respective soil depth which should be checked in studies of nitrate capture (Jama *et al.*, 1998). Secondly, during periods with downward water movement, nitrate may be leached to greater soil depths. Thirdly, nitrate may be lost through denitrification in the subsoil, a process that could itself be influenced by the presence of plant roots, since denitrification has been found to be limited by the availability of carbon rather than denitrifying microorganisms in certain subsoils (McCarty and Bremner, 1993). However, studies of denitrification in tropical subsoils and the potential influence of deep tree roots are not available.

Nitrate profiles can thus provide highly relevant information that can help to improve the nutrient cycles of agroforestry systems. However, because of the methodological problems outlined above they should usually be interpreted in largely qualitative terms. The analysis of nitrate profiles is greatly facilitated by complementary measurements of vertical and horizontal patterns of nitrogen mineralization, root distribution, soil water dynamics, and nitrogen uptake by the vegetation.

Horizontal redistribution of nutrients by trees

In addition to the capture of subsoil nutrients, trees may also cause horizontal redistribution of nutrients by taking up nutrients from an area reaching far beyond the limits of their crowns through lateral roots. Part of these nutrients is deposited in the proximity of the trees through litterfall and crown leaching. Savanna trees may possess lateral roots extending several tens of metres from the trunk (Kessler and Breman, 1991; Stone and Kalisz, 1991), and horizontal nutrient redistribution by these roots is assumed to contribute to the commonly observed nutrient enrichment in the soil under these trees (Kessler and Breman, 1991). Substantial lateral tree root extension has also been observed under agroforestry conditions: lateral roots of *Senna siamea* shelterbelts on shallow sandy soils in central Togo extended 20–30 m from the trunk, which was more than twice the tree height (Schroth *et al.*, 1995a), observations on isolated *Parkia biglobosa* trees at three sites in Burkina Faso found substantial rooting up to 10 m from trees, which was almost 3 m beyond their mean crown radius (Tomlinson *et al.*, 1998), and roots of *Senna siamea*

and *Dactyladenia barteri* in a hedgerow intercropping experiment in the humid forest zone of Nigeria extended 15 m and 5 m from the trees, respectively (Hauser, 1993). Narrow alleys between hedgerows in hedgerow intercropping are usually entirely permeated by tree roots. This led to the observation that favourable tree effects on soil nutrient status may often have been overestimated in agroforestry experiments with relatively small plots adjacent to one another, because roots from trees in treatment plots may have taken up nutrients from neighbouring control plots that were supposed to be a sole crop (see Section 3.2).

In many cases, horizontal redistribution of nutrients by trees cannot be seen as a positive effect of trees like vertical nutrient capture, because although it leads to higher tree growth and eventually higher yields of crops that profit from nutrient enrichment under the trees, this is done at the expense of neighbouring areas, which may include sheltered crop fields and pastures or even the neighbouring farm (van Noordwijk *et al.*, 1996). In other situations, however, trees may be able to capture nutrients from niches that would not be exploited by crops, such as stream banks, sites of old anthills where phosphorus has accumulated, or areas where livestock have been housed or home compounds, and to redistribute them into crop plots.

Both the vertical and the horizontal capture of nutrients by trees are poorly quantified processes and, in view of their potential importance for nutrient cycling in agroforestry, quantitative studies on both of them are required. Of particular interest is how system design, species selection and management can be combined to increase vertical nutrient pumping and reduce lateral nutrient scavenging from crop fields by agroforestry trees (Schroth, 1995, 1999).

8.2 Tracer Methods for Nutrient Uptake

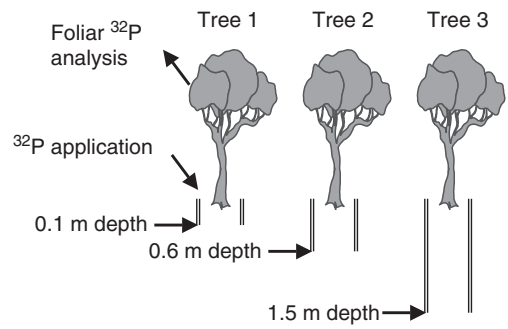
The soil volume from which roots are taking up nutrients can be determined in two ways: (i) by measuring the nutrient depletion in the soil, and (ii) by labelling certain areas in the soil with tracers and measuring the uptake into the plant. The first approach is a very crude measurement, because other processes like mineralization and leaching occur simultaneously with uptake, and nutrient depletion in a certain soil volume thus cannot be directly explained by nutrient uptake. Additionally, nutrient uptake by different plants in mixed cropping systems cannot be measured separately.

The second approach provides the possibility of directly measuring the nutrient uptake by a plant from a certain soil volume such as at a certain depth in the subsoil or at a certain lateral distance from a tree, relative to the uptake from a reference soil volume such as the topsoil or

the soil close to a tree. Root activity cannot be measured as an absolute amount of nutrient taken up per unit soil volume, but only as a so-called root activity distribution. The central assumption is that the tracer (for example, ^{32}P) is taken up in all plant parts in the same proportion as the corresponding unlabelled nutrient (total phosphorus) (IAEA, 1975).

Measurement of the vertical distribution of nutrient uptake

A relative measure of the nutrient uptake from different soil depths by a plant can be obtained by applying a point source of a tracer at different depths through a tube. For each depth, the tracer has to be applied to different plant individuals, and relative nutrient uptake is calculated from the concentration of the tracer in tissue samples of these plants (Fig. 8.3). Several tubes should be installed for each plant to label the soil as homogeneously as possible and to raise the signal in the leaves to a level that can be conveniently measured. It is evident that a completely uniform labelling cannot be achieved with this method. Therefore, the total uptake per unit soil volume cannot be calculated, as mentioned above, but different depths can be compared and the uptake from one depth in relation to the uptake from all labelled depths can be calculated. If measurements are



Tree species		Tree 1 0.1 m	Tree 2 0.6 m	Tree 3 1.5 m	Sum
Bactris gasipaes	(cpm g^{-1})	157	81	384	622
	(% of sum)	25	13	62	100
Theobroma grandiflorum	(cpm g^{-1})	2413	2	83	2498
	(% of sum)	97	0	3	100

Fig. 8.3. Schema of the application of a ^{32}P tracer at three depths to different tree individuals and calculation of relative uptake per depth for two Amazonian tree crop species. Radioactivity in leaf samples of the trees is given as cpm g^{-1} dry matter (after Lehmann *et al.,* 2001b).

carried out with individual trees, the tubes should be installed in a concentric ring around the tree, because uptake may vary between different distances from and sides of a tree (IAEA, 1975). The International Atomic Energy Agency (IAEA, 1975) arbitrarily chose 16 tubes per tree and found a high coefficient of variation (30–157%) for the root activity patterns of various tropical tree crops. Increasing the number of tubes to 60 per tree did not reduce this variability (with the exception of coconut palms).

The following tracer materials can be used in nutrient uptake studies: stable isotopes such as ^{15}N, radioisotopes such as ^{32}P or ^{33}P, and rare elements such as strontium, lithium or rubidium.

Radioisotopes

The use of radioisotopes in studies of root activity in tree-based production systems has recently been reviewed by Wahid (2001). In most of the published experiments for determining root activity patterns ^{32}P was used as a tracer. The tree species investigated included tropical and temperate fruit trees and fibre crops as well as leguminous service trees (Table 8.1). Phosphorus has two advantages over other tracers. First, it is relatively immobile in the soil and therefore remains at the point of application. Secondly, double labelling with ^{32}P and ^{33}P offers the possibility of comparing the phosphorus uptake from two different depths or distances on the same plant, thus considerably reducing the variability of the results compared with a design where the same tracer is applied at different depths or distances under different plant individuals (IAEA, 1975). ^{32}P is quite cheap but ^{33}P is expensive. Depending on the depth of application and nutrient uptake, 2–5 mCi should be used for one tree. The radioisotope should be applied together with about 5 mg of unlabelled phosphorus (as 1000 ppm P solution) to prevent a large part of the tracer phosphorus becoming fixed to oxides in highly weathered soils and hence unavailable to the target plant. Leaf sampling should be done as soon as an interpretable signal can be detected to avoid the decreasing pool size of the tracer affecting its uptake. The plant material is usually digested using heat and acid treatments, and the radioactivity is determined with a scintillation counter (IAEA, 1975). A disadvantage of radioactive materials is that they are a health hazard so transport regulations may seriously complicate their use.

As mentioned above, a further disadvantage of tracer techniques with radioactive phosphorus is the high variability of the results. Coefficients of variation between replicate trees of 100% or higher for single-labelling experiments and of 50% or higher for double-labelling experiments are not uncommon (IAEA, 1975). The high variability of data obtained with the single-labelling approach is largely an effect of confounding variability

Table 8.1. Maximum investigated root-activity distribution of several tree species using ^{32}P.

Species	Region	Depth	Distance	Reference
Cocoa (*Theobroma cacao*)	Ghana	0.45	1.52	Ahenkorah, 1975
Cocoa (*Theobroma cacao*)	India	0.90	1.50	Wahid *et al.*, 1989
Apple (*Malus domestica*)	UK	0.90	1.50	Atkinson, 1974
Cotton (*Gossypium hirsutum*)	California	1.83	1.02	Bassett *et al.*, 1970
Mango (*Mangifera indica*)	India	0.90	3.60	Bojappa and Singh, 1974
Coffee (*Coffea arabica*)	Kenya	1.80	1.35	Huxley *et al.*, 1974
Coffee (*Coffea arabica*)	Colombia	0.90	1.20	IAEA, 1975
Coffee (*Coffea arabica*)[a]	Costa Rica	0.45	2.18	Saiz del Rio *et al.*, 1961
Guava (*Psidium gujava*)	India	0.90	3.60	Purohit and Mukherjee, 1974
Gliricidia sepium	India	1.20	2.00	Vasu *et al.*, 1994
Orange (*Citrus sinensis*)	Spain	0.90	3.00	IAEA, 1975
Banana (*Musa* sp.)	Uganda	0.60	1.60	IAEA, 1975
Citrus sp.	Taiwan	0.60	2.00	IAEA, 1975
Coconut (*Cocos nucifera*)	Philippines	0.60	4.00	IAEA, 1975
Coconut (*Cocos nucifera*)	Sri Lanka	0.60	3.00	IAEA, 1975
Oil palm (*Elaeis guineensis*)	Malaysia	0.60	4.00	IAEA, 1975

[a]Determined with radioactive ^{86}Rb.

between tree individuals with variability in nutrient uptake from different soil depths, because uptake ratios between depths have to be calculated with data from different trees. This variability cannot be interpreted in a meaningful way. In contrast, the variability of the double-labelling method is caused by: (i) the heterogeneity of root-activity distribution between trees of the same species; and (ii) the spatial heterogeneity of the root activity of a tree at a given soil depth. Both of these are relevant research topics. For example, the spatial heterogeneity of tree root activity determines the 'mesh size' of safety nets against nutrient leaching (see Section 7.1).

Stable isotopes

Stable isotopes like ^{15}N have only rarely been used for root-activity measurements of annual crops (Gass *et al.*, 1971; Menezes *et al.*, 1997) or trees (IAEA, 1975; Atkinson *et al.*, 1978; Rowe *et al.*, 1999; Lehmann *et al.*, 2001b). The main obstacle for using ^{15}N is the mobility of nitrate, which is rapidly formed from the applied nitrogen source due to the high nitrification rates in many tropical soils. This may not be an important problem in acid soils with variable-charge clays and high oxide contents, which possess a high anion exchange capacity in the subsoil so that the mobility of nitrate is reduced (see Box 8.1). The mobility of the applied ^{15}N in the soil can also be reduced by applying the tracer together with a labile carbon source like sucrose which provokes microbial growth and immobilization of the nitrogen near the point of application (Rowe *et al.*, 1999). The uptake of ^{15}N is very fast and leaf samples can be collected for analysis 2–4 weeks after the application. The root activity distribution of several tropical fruit trees measured with ^{15}N was very similar to that measured with ^{32}P (Lehmann *et al.*, 2001b), confirming earlier results from apple trees (Broeshart and Netsinghe, 1972). In the former study, ^{15}N was better suited for root activity measurements than ^{32}P, because it showed a lower variability between replicates, probably due to the labelling of a larger soil volume (Lehmann *et al.*, 2001b). Preconditions for the successful use of ^{15}N in nutrient capture studies may include high microbial activity, high oxide contents, acid soil conditions and little leaching. Due to the large dilution of nitrogen in plant and soil, however, highly enriched ^{15}N sources have to be used, which are very expensive. The application of about 1–2 g ^{15}N in excess of natural abundance is recommended, preferably at 10 atom% ^{15}N excess or higher. The measurements are usually done by dry combustion coupled with mass spectrometry (Barrie *et al.*, 1995), using samples as small as 5–15 mg.

Rare elements

An alternative to isotopic tracers is the use of rare elements. Strontium, which is chemically related to calcium and magnesium, is readily available, non-hazardous, inexpensive and easy to analyse by atomic absorption spectrometry. Furthermore, strontium is more stable in soil than nitrate and does not decay like the radioisotopes. The relation of strontium and calcium in the biomass of crop plants was similar to the relation of exchangeable strontium and calcium in the soil in which the plants were grown (Menzel and Heald, 1959). Fox and Lipps (1964) measured similar root-activity patterns of lucerne (*Medicago sativa*) with strontium and ^{32}P. Van Rees and Comerford (1986) measured the uptake of strontium by

pines (*Pinus elliottii*) and understorey vegetation from different soil depths. The drawback of this method is its low sensitivity. Also, if large amounts of strontium have to be applied, it may cause nutrient uptake disorders and growth depression of the plants (Fox and Lipps, 1964). Rubidium and lithium are chemically related to potassium, but are rarely used as tracers because the former is expensive and the latter is phytotoxic. An exception is the work by Fitter (1986).

Measurement of the lateral distribution of nutrient uptake

For the measurement of lateral nutrient uptake, i.e. from different distances from the plant, the same techniques can be used as for the nutrient uptake from different depths using strip or point applications of ^{32}P (IAEA, 1975; Wahid, 2001), ^{15}N (Atkinson *et al.*, 1978) or strontium (Lehmann *et al.*, 1999b). The tracers can either be applied at a certain depth or on the soil surface, and this offers the possibility of labelling whole areas (Lehmann *et al.*, 2000). This approach allows the calculation of uptake per unit area, either relative to the amount of labelled fertilizer applied to this area or relative to a reference area. If the area to be labelled is large, stable isotopes are preferable to radioisotopes because of the lack of health hazards and over-rare elements because of the smaller amounts needed. If the above-ground biomass can be determined, the total recovery of the applied source can be calculated, which may be more useful information than root-activity comparisons between distances. Additionally, the relative importance of different areas for nutrient uptake can be compared, for example beneath the hedgerow and in the alley between hedges in hedgerow intercropping experiments (Lehmann *et al.*, 2000).

With all tracer applications, care has to be taken to maintain sufficient distance between treatments in order to avoid cross-contamination. This is especially true for radioisotopes because of the high sensitivity of the measurement.

Tracer techniques for measuring root-activity distribution have certain advantages compared with root distribution studies and can, therefore, be a useful supplement to these. Tracers are only taken up by active roots, which are often difficult to distinguish visually from dormant or dead roots. They can also be used to separate nutrient uptake by different species from the same soil volume, whereas the visual distinction of roots from different species is often difficult (see Section 12.3).

Chapter 9
Nutrient Exchange with the Atmosphere

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9.1 Synopsis

Vegetation exchanges nutrients with the atmosphere through several processes, some of which can be influenced by agroforestry techniques. In this chapter, the effects of trees on nutrient gains through atmospheric deposition and nutrient losses through fire are outlined. Biological nitrogen fixation is another important atmospheric exchange, which is covered in Chapter 13.

Nutrient gains from atmospheric deposition

Atmospheric deposition of nutrients occurs either in a wet form associated with rain or fog, or in a dry form as particles or gases. Estimates of atmospheric inputs for different tropical sites can be found in Bruijnzeel (1991) and Szott *et al.* (1991), who concluded that annual inputs are in most cases low, although substantial quantities of nutrients may be deposited over long time spans. A critical review of atmospheric phosphorus inputs has been provided by Newman (1995), who points to methodological and other error sources in published values. Atmospheric nitrogen inputs for West Africa are given by Robertson and Rosswall (1986) as part of a regional nitrogen balance. For sub-Saharan Africa, Smaling *et al.* (1993) reported the following linear relationships between wet deposition of nitrogen (N), phosphorus (P) and potassium (K) in kg ha^{-1} $year^{-1}$ and the square root of average rainfall (r) in mm $year^{-1}$:

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$$N = 0.14r^{0.5} \quad (9.1)$$

$$P = 0.023r^{0.5} \quad (9.2)$$

$$K = 0.092r^{0.5} \quad (9.3)$$

The presence of trees in agroforestry systems can influence atmospheric nutrient deposition by rain through the interception of rainfall and modification of the wind field (D.M. Smith *et al.*, 1997a). Tree rows may increase rainfall and corresponding nutrient inputs on the windward side and reduce them on the leeward side (Wallace, 1996). Trees with high stemflow, such as certain palm species, concentrate atmospheric water and nutrient inputs in the proximity of their stem (Schroth *et al.*, 1999b, 2001a).

Dew formation occurs mainly on leaf surfaces exposed to clear sky and is, therefore, influenced by trees through the amount of leaf area they support and the orientation and angular distribution of leaves. Condensing dew dissolves hygroscopic materials on the leaf surface that may have originated from dry-deposited aerosols (Burkhardt and Eiden, 1990). The deposition of water-soluble gases such as ammonia or nitric acid is enhanced by dew (van Hove *et al.*, 1989), although the stable atmospheric conditions generally occurring when dew is formed may limit the effectiveness of this mechanism. The chemical composition, especially the pH, of dew is determined by dissolved materials including ions leached from the leaf, and this may influence gas dissolution. Revolatilization and denitrification of dissolved components may occur as dew dries (Takenaka *et al.*, 1999).

In contrast to deposition of pure water as dew, fog is deposited as liquid water already including dissolved substances. Fog droplets are deposited by impaction and interception, and the leaves of trees often act as very effective fog collectors, especially in mountainous regions where clouds are directed by wind towards hills (cloud-stripping; Doumenge *et al.*, 1995). In some cloud-forest areas, fog interception is an important water source (Cavelier, 1996) and would also be expected to contribute significantly to atmospheric nutrient deposition. In the northern Andes, a cloud belt is located at 1000–1400 m. In other regions, the altitude of such zones of elevated atmospheric humidity varies with climatic and geographical factors, such as general humidity and distance to the sea (Cavelier, 1996).

Dry deposition includes nutrients in dust and aerosols. These may be transported over long distances, such as from the Sahara to the West African savanna and rainforest zones by the Harmattan winds, or may come from local sources, such as soil and vegetation. In Niger, average dust inputs from the Harmattan of 1500 kg ha^{-1} $year^{-1}$, containing 2.7 kg of calcium, 0.9 kg of magnesium and 1.2 kg of potassium, were measured

with open-bucket samplers. The dust contained 10–30 times more exchangeable calcium, magnesium and potassium than the soil on which it was deposited, and the cation exchange sites of the dust particles were base saturated, whereas the topsoil was only 20–30% base saturated. The dust input could thus have a significant effect on soil chemistry (TropSoils, 1989). With increasing distance from the Sahara, the annual dust deposition generally decreases; however, a deposition of 2.5 kg ha^{-1} $year^{-1}$ of potassium and 3.5 kg ha^{-1} $year^{-1}$ of calcium was still measured in the Taï forest in the south-western Côte d'Ivoire in another study (Stoorvogel *et al.*, 1997). This dust is low in nitrogen due to its Saharan origin (Robertson and Rosswall, 1986). Plant pollen is a source of dust that is relatively rich in phosphorus (Newman, 1995).

The presence of trees strongly increases the deposition of dust and aerosols by filtering them from the air with their crowns (Stoorvogel *et al.*, 1997). Deposition of dust on trees, from which it is washed down by subsequent rains, could contribute to higher clay and nutrient contents in the soil under savanna trees (Sanchez, 1987; Young, 1997), although there are numerous alternative explanations, such as preferential establishment of trees on more fertile sites, vertical and lateral redistribution of nutrients by tree roots, animal droppings under trees, and differences in soil erosion between areas with and without tree cover. Wind deposition may be uneven over short distances and contribute to soil heterogeneity. Adderley *et al.* (1997), for example, reported surface sand derived from lacustrine and aeolian deposits, varying from 0 to 690 mm in depth over clay soils across a 30 ha site in northern Nigeria, with a mean change of 0.46 mm m^{-1} from north to south attributable to airborne deposition. Tree survival at this site was highly correlated with the sand content of surface soil.

Effects on the wind field and thus on rain distribution and dust interception are greatest for large trees. Pronounced effects would be expected from windbreaks, boundary plantings and perhaps from large solitary trees in crop fields, pastures or shaded tree crop plantations. Small, regularly pruned trees would not be expected to have significant effects on atmospheric deposition (Szott *et al.*, 1991).

Nutrient losses with fire

Agroforestry practices may also affect carbon and nutrient exchange with the atmosphere by reducing the frequency of fires. Fire is a common management tool in traditional agriculture in both forest and savanna ecosystems. It serves for site clearing, transformation of biomass into nutrient-rich ash, weed and pest control, pasture regeneration and hunting. Burning early in the dry season can prevent much more intensive and destructive fires in the late dry season. In simultaneous agroforestry

practices, the presence of the trees may be an important incentive for farmers to protect their land from spontaneous fires, especially in savanna areas. Belts of fire-resistant trees under which the ground vegetation is suppressed and litter periodically burned are an agroforestry technique that can help to prevent the spread of bush fires. Improved fallows should also not be burned before cropping, especially when the nitrogen accumulation in legume biomass is an objective of the fallowing, although farmers may prefer to burn to facilitate site clearing (MacDicken, 1991).

During fires, nutrient losses to the atmosphere occur through volatilization. In addition, ash particles are carried away by wind and the chimney effect generated by the fire (Mackensen *et al.*, 1996). Fire accounts for the bulk of all nitrogen losses from West Africa (Robertson and Rosswall, 1986), and empirical evidence suggests that ecosystems affected by recurrent burning as occurs in savannas are characterized by relatively low nutrient stocks (Ehrlich *et al.*, 1997). In addition to the nutrient losses, the release of the greenhouse gases carbon dioxide, carbon monoxide, methane and nitrogen oxides as well as aerosols during the combustion process has drawn the attention of researchers concerned about climate change to vegetation fires in the tropics (Carvalho *et al.*, 1995; Kauffman *et al.*, 1998).

During the burning of two secondary forests and one fallow site in eastern Amazonia, Mackensen *et al.* (1996) measured the following element losses: 95–98% of the nitrogen in the biomass, 67–76% of the sulphur, 27–47% of the phosphorus, 16–48% of the potassium, 17–43% of the magnesium, 9–35% of the calcium and 17–30% of the sodium, together with 94–98% of the carbon. The stems were removed from the forest sites before the burn, leaving only relatively small material, which explains the high carbon and nitrogen losses. Where tree stems are burned, lower combustion efficiencies are achieved (Carvalho *et al.*, 1995; Kauffman *et al.*, 1998). When burning secondary forests in Brazilian Amazonia, Hughes *et al.* (2000) found that the average proportions of the nitrogen, sulphur, phosphorus, potassium and calcium in the above-ground biomass that were lost from the site through fire were 70, 54, 33, 17 and 20% of the pre-fire pools. Kauffman *et al.* (1998) identified three factors influencing nutrient losses from vegetation fires: (i) the level of biomass consumption (nutrient losses increase with increasing combustion efficiency); (ii) nutrient distribution within biomass classes with varying susceptibility to combustion losses (lower nutrient losses from stems than from fine litter); and (iii) temperatures of volatilization of different elements (greater losses for nitrogen than for phosphorus, potassium and calcium). Effects of fire on soil properties have recently been reviewed by Neary *et al.* (1999).

9.2 Methods for Atmospheric Nutrient Inputs

Wet deposition of nutrients at a site can be approximated by collecting rainfall in an open area or above the canopy with funnels and multiplying the periodical rain totals with the corresponding nutrient concentrations (Bruijnzeel, 1991). Bias can result from strong winds during rainfall leading to edge effects which affect collector yields, or from dry-deposited material in the collectors including dust, vegetation debris or insects. Coarse material may be prevented from entering the collectors by grids or ping-pong balls. If funnels are used, adhesion of water to the surface may lead to underestimation of water inputs, especially for small rains.

The determination of dry deposition to an ecosystem poses more difficulties, as this term summarizes a wide range of particle size classes from gas molecules to dust particles, extending over five orders of magnitude and therefore involving considerably different deposition mechanisms. Whereas for dust particles (>10 μm in diameter) gravitational settling is dominant, the deposition of smaller aerosol entities is dependent on characteristics of the acceptor, such as geometry, microroughness and chemical composition. For the latter group, artificial samplers will not give realistic results in most cases. Four approaches to the determination of dry deposition are touched upon below.

Micrometeorological methods

Micrometeorological measurements such as eddy correlation are most effectively used for gases. They require very specialized and cost-intensive equipment and mostly have homogeneous terrain as a precondition, which makes them unsuitable for heterogeneous agroforestry conditions (Finkelstein and Sims, 2001).

Washing of plant surfaces

Leaves, twigs or whole trees have been washed with water to quantify atmospheric inputs (Reiners and Olson, 1984). Washing procedures of leaves may answer specialized questions regarding aerosol and gas deposition, but the dependence of leaf orientation and microsite within the canopy may be decisive for the amount of deposited material, so extensive data sets are needed to obtain a complete picture. Furthermore, the efficiency of washing depends on the age of the leaf, and there are effects of leaching, which will mostly be dependent on the washing time. Leaf washing may be especially useful if leaves of different, neighbouring

trees are compared, which may have different sampling characteristics for dry-deposited material.

Artificial filters

Artificial filters for dry deposition of nutrients can be calibrated against natural foliage and exposed in different positions of tree canopies (Kellman and Carty, 1986). As in the method discussed before, the effort for measuring inputs in different crown positions may be considerable.

The canopy balance method

This method has been described by Ulrich (1983) and Lovett and Lindberg (1984). It consists of the comparison between rainfall, which is sampled above the canopy or on a nearby location without trees, and throughfall and stemflow, sampled within the canopy, assuming that these contain the substances that were dry deposited on the canopy before the respective rain event. Much less equipment than in micrometeorological measurements is needed and aerodynamically homogeneous conditions are not required to obtain a time-integrated overall picture of dry deposition.

However, nutrient deposition in throughfall and stemflow under trees would correspond to total atmospheric deposition only if it could be assumed that: (i) uptake of deposited nutrients through the leaves is negligible in comparison to the deposited quantities; and (ii) there is negligible leaching of root-absorbed nutrients from the canopy (Cape, 1993). These assumptions are usually not correct. Besides the nutrients derived from atmospheric deposition, throughfall and stemflow contain nutrients that were taken up by the trees from the soil and were leached by the rainwater from foliage and bark. This latter part reflects mere recycling of nutrients, similar to much of the nutrients in litterfall. Furthermore, certain elements such as hydrogen and ammonium ions are taken up from throughfall water by the plants during crown passage and are replaced by other cations (especially calcium, magnesium, potassium and manganese), which are leached from the leaves and therefore increase in the throughfall water (Tukey, 1970; Lovett, 1992). The recent detection of microscopic waterfilms extending from the leaf surface into the stomata, through which nutrient ions may move into the leaf, indicates that foliar nutrient uptake may even continue during prolonged periods of the day when no free water is present on the leaves (Burkhardt and Eiden, 1994; Eichert *et al.*, 1998). In addition, epiphytes may retain nutrients from

atmospheric deposition and fix nitrogen, and these nutrients may be released in pulses following drying cycles (Coxson, 1991).

The canopy balance method therefore requires the separation of those nutrients in throughfall and stemflow that originate from atmospheric deposition from those that were taken up by the plant roots and were leached from the foliage. For this, sodium has been used as a natural tracer. Sodium is not leached from the surface of most plants in significant quantities, and it can thus be assumed that all the sodium on a plant surface comes from the atmosphere (Newman, 1995). Kellman and Carty (1986) measured dry deposition of nutrients in pine trees with artificial filters which were exposed in the canopy. The deposition of sodium on the filters was calibrated against that on natural foliage by exposing the filters together with prewashed pine whorls to the atmosphere and analysing leachates from filter and foliage for deposited nutrients. It was assumed that other elements were deposited at similar ratios on filters and foliage to sodium. The nutrient deposition on the trees could thus be calculated from that on the filters (Kellman and Carty, 1986).

Instead of exposing aerosol filters at different positions in a tree canopy, sodium can also be measured in throughfall and stemflow from the respective trees. With the ratio of sodium and other elements measured in dust filters, and after subtracting the sodium input from wet deposition (rainwater), the dry deposition of different elements on the tree canopy can then be estimated (Ulrich, 1983). In both cases, the assumption that other elements behave in a similar way to sodium with respect to their deposition on artificial filters and foliage is open to doubt, as different elements may be contained in aerosols and dust particles differing widely in size and surface properties (Sehmel, 1980; Newman, 1995). The methods described here can thus only provide an approximation of dry deposition. Apart from sodium, titanium has also been used as reference element assumed to have purely atmospheric origin (Stoorvogel *et al.*, 1997). The separation of atmospheric deposition from canopy leaching as a component in throughfall and stemflow has also been attempted with radioactive tracers applied to the soil (Cape, 1993).

The heterogeneity of throughfall may be considerable, and large numbers of samplers may be necessary to obtain a representative average value for an area (Draaijers *et al.*, 1996). To improve the precision of the estimate and/or reduce the number of collectors required, their positions can be changed from time to time (Lloyd and Marques, 1988). However, the spatial heterogeneity of nutrient inputs in throughfall and stemflow, as affected by agroforestry trees with different crown architectures, may also be of considerable interest itself (Schroth *et al.*, 2001a). Gaseous deposition of nutrients to the stomata is not included in measurements using the canopy balance method.

Sample collection and analysis

Rainwater, throughfall and stemflow are usually very dilute solutions that are highly susceptible to contamination both in the field, from leaves, insects, birds, mineral fertilizer, etc., and in the laboratory. Thus, collectors should be washed very carefully, and frequent collection of the samples is advisable to reduce the risk of sample loss by contamination and to prevent chemical alterations due to microbial action, affecting especially phosphorus and ammonium. Stemflow samples often contain organic particles and need to be filtered before analysis. Filtration at 0.45 μm is sometimes used to produce normalized conditions and reduce microbial activity, but filtration with prewashed paper filters is also acceptable. The inclusion of organic nitrogen and phosphorus forms in the analyses is essential, as these may contribute significantly to the total inputs of these nutrients (Schroth *et al.*, 2001a).

9.3 Methods for Nutrient Losses from Burning

According to Mackensen *et al.* (1996), three approaches to measuring nutrient losses during biomass burning can be distinguished: (i) collection of smoke samples above the fire, which are analysed for gases and ash particles; (ii) simulation of the fire in a muffle furnace (where flame conditions differ from those in the field and losses of ash particles cannot be simulated); and (iii) quantification of carbon and nutrient stocks in the biomass and ash before and after the burn in the field. Only the latter approach will be discussed here, as it is probably the most useful in agroforestry research. Losses to the atmosphere in gaseous and particulate form cannot be measured separately with this approach, but this may often not be necessary.

The method requires the estimation of the mass (often calculated from measurements of volume and density) and nutrient concentration of the biomass before and after the burn. The volume of stems and woody debris on the ground can be estimated non-destructively with the line intersect (or planar intersect) method (van Wagner, 1968; Brown and Roussopoulos, 1974). The wood particles are divided into diameter classes, and the intersections of the particles with sampling lines are counted. The total volume V of the biomass is calculated as

$$V = n\alpha\,[(\pi^2 d_q^{\,2})/8L] \tag{9.4}$$

where n is the number of intersections, d_q is the average squared diameter of the particles in a diameter class, L is the length of the sampling line, and α is the correction for non-horizontal orientation of wood particles and is defined as the average secant (= $\cos^{-1}$) of the angle of the particles with

the horizontal. Sampling bias due to non-random orientation of the wood can be minimized by using several lines placed at different directions. In an Amazonian pasture, Kauffman *et al.* (1998) used 32 systematically distributed sampling lines both before and after a burn. Wood particles were divided into the diameter classes 0–0.64 cm, 0.65–2.54 cm, 2.55–7.5 cm, 7.6–20.5 cm and >20.5 cm, and increasing lengths of the sampling lines were used for larger diameter classes (1 m for the smallest and 15 m for the largest class). Average angle and diameter were measured on 100 randomly selected particles of each size class. Samples for wood density and nutrient concentration were also collected per size class.

The preburn nutrient stocks have to be determined shortly before the burn, because nutrients may be leached from the biomass into the soil when the cut vegetation is exposed to rain before the fire, and this reduces potential losses to the atmosphere. After the burn, the nutrient stocks are measured both in the unburned residues and in the ash and charcoal. Again, measurements have to be taken immediately, as nutrients may be lost from the ash by leaching into the soil without necessarily being lost from the site, or by particle erosion. For the quantitative collection of ash, steel trays may be placed on the soil under the vegetation debris (Mackensen *et al.*, 1996), or the ash may be collected from microplots, e.g. with a vacuum cleaner (Kauffman *et al.*, 1998).

Chapter 10
Soil Structure

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10.1 Synopsis (*G. Schroth*)

Soil structure comprises the arrangement of individual particles in the soil and is especially concerned with their aggregation, the resulting pore size distribution and the stability of the aggregated state (Payne, 1988). Soil structure affects the availability and mobility of water, air and nutrients and influences the growth of plant roots, which is generally reduced in compact, dry and poorly aerated soil. By controlling the access of plants to water and nutrients, soil structure determines, to a large extent, the productivity of a site. The maintenance of an adequate soil structure is an essential element of the sustainability of land-use systems. Where land-use practices have led to structural degradation of the soil, the improvement of soil structure is often a precondition for the regeneration of site productivity. This is usually much more difficult to achieve than the correction of chemical fertility problems (Alegre *et al.*, 1986).

Structural degradation of agricultural soils

Soil structural degradation typically occurs when forest or savanna vegetation is replaced by annual cropping systems. Under continuous cropping with maize for 3 years on a coarse-textured Alfisol in the Nigerian rainforest zone, increased bulk density, reduced soil porosity, reduced water infiltration, surface crusting and reduced water-holding capacity compared with fallow soil indicated the progressive degradation of soil

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structure. The degradation was apparently caused by severe loss of soil organic matter, surface erosion and reduced earthworm activity under the annual crops (Juo and Lal, 1977). Similarly, annual cropping in the wooded savanna zone of West Africa has often led to increased bulk density and reductions in porosity, structural stability and water infiltration (Pieri, 1989). Cropped ferralitic soils in Queensland, Australia, have developed an increased tendency for surface sealing under high-intensity rainfall, associated with a decline in soil organic matter (Bell *et al.*, 1998). Degradation of soil structure has also been observed under tree crops such as cocoa (*Theobroma cacao*; Ekanade, 1985).

Soil structural degradation often begins when inappropriate land-clearing methods are used. On an Oxisol in central Amazonia, mechanical forest clearing destroyed many pores with an equivalent size of >0.1 μm in the topsoil, thereby reducing the amount of plant-available water (Chauvel *et al.*, 1991). Physical soil degradation caused by mechanized land clearing has also been described for an Ultisol in the Peruvian Amazon (Alegre *et al.*, 1986) and an Alfisol in West Africa (Hulugalle, 1994) and contributed to yield losses in the second rotation of an oil palm (*Elaeis guineensis*) plantation on a ferralitic soil in the Côte d'Ivoire (Caliman *et al.*, 1987).

Potential of trees to improve the structure of agricultural soils

Soil structure is stabilized by the presence of binding materials such as clay, if flocculated by polyvalent cations such as calcium or aluminium, lime, and oxides and oxihydroxides of iron and aluminium (Payne, 1988). The action of these binding materials is not influenced by the introduction of trees to agricultural systems and so will not be further discussed here (see also Section 10.3). However, the potential of trees to contribute to the maintenance and rehabilitation of soil structural characteristics in other ways has been well established. Soil physical characteristics have often been improved in hedgerow intercropping plots compared with control plots with annual crops (Rao *et al.*, 1998). The form of these improvements has included better soil aggregation, lower bulk density, lower resistance to penetration, reduced surface sealing, improved soil porosity and consequently higher water infiltration and water-holding capacity. Similar positive effects have been found under planted fallows. The integration of trees in agricultural systems affects soil structure through the following mechanisms.

- Trees may increase the quantity of litter or mulch on the soil surface, which prevent rain drops hitting and breaking down soil aggregates, which leads to clogging of infiltration paths. Reduced overheating and evaporation from the soil surface are added advantages of a litter layer.

In the Nigerian rainforest zone, negative effects of annual cropping on soil structure were much reduced when the soil was mulched, as this avoided crusting and maintained a higher earthworm activity (Juo and Lal, 1977; Juo *et al*., 1995; see also Chapter 16).

- Trees can increase soil organic matter levels and microbial activity through increased inputs of above- and below-ground biomass, reduced soil temperature and reduced erosion (see Chapters 4 and 17). Soil organic matter has a stabilizing effect on soil aggregates, and the loss of humus associated with cultivation is usually accompanied by structural degradation. Addition of easily decomposable organic residues to soils, such as certain tree prunings, leads to the synthesis of polysaccharides and other organic compounds that stabilize soil structure (Stevenson and Cole, 1999). Hyphae of saprophytic and mycorrhizal fungi can bind mineral and organic particles together into stable aggregates (Tisdall *et al.*, 1997), and part of the stabilizing effect of plant roots on the soil structure is due to their association with mycorrhizas (Miller and Jastrow, 1990). Microbial polysaccharides produced in the rhizosphere could also contribute to this effect (Angers and Caron, 1998).
- The introduction of trees to agricultural systems will influence the quantity and types of roots present in the soil, which may affect soil structure. Plant root systems stabilize soil structure by enmeshing aggregates, releasing binding materials (mucilage) into the rhizosphere (Morel *et al.*, 1991) and increasing soil microbial activity. As a result, root length or mass may correlate significantly with soil aggregation (Miller and Jastrow, 1990). When roots grow through the soil, they enlarge existing pores and create new ones, but their radial pressure may, at the same time, compress the soil around these pores. Water uptake by roots also affects the formation and fragmentation of aggregates by influencing wetting–drying cycles in the surrounding soil (Angers and Caron, 1998). The relatively large, continuous macropores resulting from root growth can play an important role in soil aeration and rapid macropore flow of water through the soil (see Chapter 11). In addition, old root channels are often used by new roots, which thereby avoid the mechanical resistance of the soil matrix and may profit from the more favourable chemical environment provided by root debris (van Noordwijk *et al.*, 1991a). Plants differ in their ability to penetrate compact soil, and the increased soil porosity caused by plants with strong root systems, including certain tree species, could improve the root development of associated or subsequent crops with weaker root systems and thereby their access to additional soil water and nutrients. Like other macropores, such biopores could be especially important in facilitating root penetration when the soil matrix is too dry or its strength too high to allow rapid

root growth. This can be essential for the establishment of seedlings (Cornish, 1993). This biological drilling by plant roots (and soil fauna) is most important at sites with compact subsoil horizons, and trees and woody shrubs are especially effective because of their perennial nature and their known ability to penetrate very hard horizons. However, in their critical review of the subject, Cresswell and Kirkegaard (1995) point out that there is not always a clear causal relationship between crop yield increases in rotations with deep-rooting species (both herbaceous and woody) and the creation of macropores in compact subsoil. To demonstrate biological drilling as the reason for yield improvements under these conditions, it would be necessary to separate effects of improved subsoil structure from those of altered soil chemical properties or the carry-over of root diseases. In future studies of the topic, particular attention should be given to the hydrological conditions of roots growing in existing macropores as influenced by possibly incomplete root–soil contact (e.g. small crop roots growing in wide tree root channels) as well as the possible concentration of pathogenic microorganisms and nematodes in such biopores (Cresswell and Kirkegaard, 1995). Methods of studying interactions between roots and soil structure have been reviewed by van Noordwijk *et al.* (1993b).

- Through the quantity and quality of root and shoot litter, microclimatic effects and avoidance of soil tillage trees may influence the abundance, composition and activity of the soil fauna, such as earthworms, termites and ants, which in turn may affect soil structure through their burrowing activity. Faunal effects on soil structure are further discussed in Chapter 16.

These strongly interdependent mechanisms deserve the attention of agroforestry researchers because they provide a basis for the use of trees in the management of soil structure. A quantitative understanding of these mechanisms is necessary for the identification of criteria for agroforestry tree species that are efficient in the maintenance and regeneration of soil structure. On a sodic soil in India, improved soil physical conditions under *Prosopis juliflora* compared to *Acacia nilotica* was explained by the higher litterfall and, more importantly, the larger root system of *P. juliflora* (Garg and Jain, 1992). A comparison of nine leguminous tree species and a spontaneous control in central Côte d'Ivoire provided indications that fallow species with high root mass are more efficient in the rehabilitation of compacted soils than species with lower root mass (Schroth *et al.*, 1996). Afforestation of grassland in the Philippines with *Acacia auriculiformis* improved topsoil physical characteristics and water infiltration, whereas *Pinus kesiya* did not have this favourable effect, apparently because of the high faunal activity and well-developed root system of *A. auriculiformis* as

opposed to the dense fungal mycelia in the soil associated with *P. kesiya* (Ohta, 1990). More mechanistic studies relating tree characteristics to their effect on soil structure are clearly desirable.

10.2 Methods for Soil Bulk Density (*W.G. Teixeira, B. Huwe*)

Bulk density is a simple measure of soil structure. It is defined as the ratio of the mass of an oven-dry soil sample (dried at 105°C to constant weight) to its bulk volume. It is a temporally and spatially variable soil property that can be used as an indicator of changes in soil structure caused by agricultural management, root growth and activity of soil flora and fauna. In this section, only field sampling procedures will be discussed. For detailed laboratory procedures see Blake and Hartge (1986) and McIntyre and Loveday (1974).

Examples of agroforestry studies in which different plant species and management practices caused significant changes of bulk densities in topsoil and subsoil can be found in Torquebiau and Kwesiga (1996), Mapa and Gunasena (1995) and Huxley *et al.* (1994). A reduction of soil bulk density is frequently interpreted as an improvement of soil physical properties, which is in most cases appropriate. Nevertheless, a reduction in bulk density (and increase in porosity) can sometimes be disadvantageous, for example when increased water infiltration and percolation cause increased nutrient leaching. Also, the plant-available water capacity of coarse-textured soils may be lower at low than at high bulk density, and can then be increased by compacting the soil (Archer and Smith, 1972).

Core method

The soil sample is collected by driving a steel cylinder into the soil to the desired depth. The cylinder is then carefully removed to obtain an exact volumetric sample. Sampling is normally carried out by hammering or jacking. Especially for soils with large silt and clay content it is important to use appropriate samplers to avoid compaction of the sample (McIntyre and Loveday, 1974). The core diameter should be at least 7.5 cm and preferably 10 cm if accuracy is critical, and the core length should ideally be equal to the diameter (McIntyre, 1974). Sampling should be carried out at intermediate soil moisture conditions because sampling during very wet conditions may cause compression of the sample, and sampling during very dry conditions may result in shattering of the cores. In swelling soils,

bulk density depends on soil moisture, and bulk density data should thus either be accompanied by moisture data at the time of sampling or, preferably, be determined at a specified matric suction. A suitable moisture content for measuring bulk density in such situations is when lateral swelling has reached a maximum and all cracks are closed (McIntyre, 1974). If the aim of the sampling is only the measurement of bulk density, it is not necessary to keep the collected samples undisturbed or to avoid soil moisture loss.

Clod method

In situations where the sampling of cores is not practicable, as in gravelly soils, bulk density can be measured using soil clods. For such soils it is generally better to make measurements on a large clod with small disturbance than on several small cores that may have undergone serious disturbance during collection (McIntyre, 1974). The two available techniques for measuring the bulk density of soil clods, paraffin wax coating and kerosene saturation, are only suitable for relatively stable clods. These techniques apply Archimedes' principle to determine clod volume by weighing the clod in air and in a liquid of known density. Particularly for expansive soils, this method can lead to an underestimation of bulk density due to expansion of the clod when restraining forces caused by the overlying soil are removed (Blake and Hartge, 1986). Expansive soils must therefore be equilibrated with a standard matric suction for comparison of their densities (e.g. 100 hPa; McIntyre and Loveday, 1974).

Other methods

Field methods suitable for gravelly soils involve the excavation of a quantity of soil for drying and weighing and the determination of the volume by filling the hole with sand or placing a rubber balloon in it which can then be filled with water (Blake and Hartge, 1986; Liu and Evett, 1990). Nuclear techniques are also available but their use is common only in engineering applications.

10.3 Methods for Aggregate Stability (*M. Grimaldi*)

The aggregate stability of soil affects infiltration of water and susceptibility to water erosion, crust formation, hardsetting and compaction (Angers and Mehuys, 1993). A large number of methods for evaluating the aggregate stability of soils have been proposed since the pioneering work

of Yoder (1936). These testify, on the one hand, to the importance of this soil characteristic, and demonstrate, on the other hand, the difficulty of defining a universally applicable method. This difficulty arises because of the diversity of factors and mechanisms of disaggregation which act on different organizational levels of soil structure, and which should be reflected by the measurement. The principal mechanisms of disaggregation are slaking of aggregates by the compression of entrapped air, disaggregation by differential swelling of clays which provokes a microfissuration of the aggregates, disaggregation by the impact of rain drops and physicochemical dispersion.

The stability of aggregates depends on their mineral and organic constituents (Emerson and Greenland, 1990). Three types of constituents are particularly important: the percentage of exchangeable sodium (Shainberg, 1992), the oxides and oxihydroxides of iron and aluminium (Le Bissonnais and Singer, 1993), and soil organic matter (Chenu, 1989; Haynes and Swift, 1990). Of these, it is mainly the dynamics and distribution of soil organic matter that can be influenced by trees (see Section 10.1).

A method of measuring aggregate stability that has recently been proposed by Le Bissonais (1996) integrates key aspects of the older methods while being adapted to a wide range of different soil types. It consists of three main treatments which simulate the three principal mechanisms of aggregate fragmentation:

- treatment 1: disaggregation by slaking, provoked by rapid immersion in water;
- treatment 2: disaggregation by microfissuration, provoked by slow capillary wetting;
- treatment 3: mechanical disaggregation, provoked by shaking in water after slow capillary wetting.

The initial physical conditions of the samples, and especially their moisture and the size of the aggregates, have an important influence on the results of the treatments. These conditions are therefore standardized to allow the comparison between different samples, e.g. for different pedological horizons, climatic conditions or cropping systems. The specific soil characteristics such as their water content at the time of sampling and the plot history are taken into consideration when interpreting the results.

The soil samples are sieved to obtain aggregates of a size between 3 and 5 mm, followed by air-drying or equilibration at a certain matric potential. For treatment 1, the aggregates are immersed for 10 min in distilled water. This treatment can also be used as a qualitative, simple and rapid field test. For treatment 2, the aggregates are placed on a filter paper that is wetted with distilled water, and for treatment 3 they are wetted with ethanol and then manually shaken in distilled water. Wetting in ethanol

reduces the slaking of the aggregates by reducing the speed of the water penetration and the swelling of the clay minerals. For each treatment, and thus for each of the three mechanisms of disaggregation, the distribution of the aggregates in seven size classes is determined (>2 mm, 2–1 mm, 1–0.5 mm, 0.5–0.2 mm, 0.2–0.1 mm, 0.1–0.05 mm and <0.05 mm). The detailed protocol is given by Le Bissonais (1996), who proposes synthesizing the results of the measurement by calculating the weighted mean diameter of the aggregates, which is the sum over all size classes of the product of the dry weight of the aggregates and the mean between the two limits of the respective size class.

If physicochemical dispersion is the main mechanism of disaggregation in a soil, the three treatments lead to the same distribution of aggregate sizes with a large fraction <0.05 mm. In this case it is recommended to evaluate the quantity of dispersed clay in the suspension that is produced by one of the treatments. If, on the other hand, the disaggregating effect of all the three treatments is small, a treatment proposed by Hénin *et al.* (1958) may still reveal differences between samples. It consists of wetting the aggregates in benzene before the immersion in water. Benzene increases the wettability of the pore walls and can therefore reveal the hydrophobic effect of certain organic molecules.

An approach to evaluating the causal relationships between aggregate stability and correlated soil characteristics such as root length or length of fungal hyphae in a sample has been described by Jastrow and Miller (1991). These authors also provide a very useful discussion of the measurement of aggregate stability as related to biological soil processes.

10.4 Methods for Soil Porosity and Pore Size Distribution (*M. Grimaldi*)

The measurement of the parameters of the pore space of a soil, such as total porosity, pore size distribution and morphology of the pores, is a quantitative, indirect approach to the analysis of soil structure. The soil pores are discontinuities that affect the spatial distribution of mineral and organic soil constituents and allow us to distinguish between successive levels of organization of these constituents within the soil: clay domains or microaggregates, aggregates, clods and horizons. It is always important, but especially so in agroforestry situations, to consider the lateral variations of soil porosity as much as its vertical variations in the soil profile. Within a single soil horizon, aggregates or soil volumes differing widely in porosity can coexist, depending on the spatial patterns of faunal activity, root distribution or tillage practices.

Soil porosity

The porosity of a soil sample (n) is the volume of pores related to the total volume of the sample. It is calculated from bulk density (γd) and particle density (γs), both measured in g cm^{-3}:

$$n = 1 - \frac{\gamma d}{\gamma s} \tag{10.1}$$

For the measurement of bulk density γd see Section 10.2. The particle density γs can be measured with submersion or pycnometer methods (Blake and Hartge, 1986). In mineral soils it is often assumed to be 2.65 g cm^{-3}, the value for quartz and also kaolinite. However, this assumption can lead to errors for soils with high contents of organic matter (which reduces γs) or iron (which increases γs). For example, for a ferralitic soil in Amazonia, γs was 2.64 g cm^{-3} with 0.8% organic carbon and 2.52 g cm^{-3} with 4.3% of organic carbon. High iron contents – recognizable by the red soil colour – can increase γs to values close to 3 g cm^{-3}.

If the inspection of the soil profile reveals several more or less homogeneous units, such as horizons or subhorizons, samples for porosity measurements should be collected from each of these units. The necessary number of replicate samples for porosity measurements (as for any other measurement) depends on the required precision and variability of the porosity between individual samples (see Section 3.2), which vary widely between different field situations. Although it is not possible to define a priori the required number of replicate samples, five replicates per sampling unit should usually be considered a minimum when bulk density is determined with cylinders (100 cm^3 or larger) or clods. Whether the sampling is carried out in a random pattern or on a predefined sampling grid, it is important for the interpretation of the measurements and their variability to note the visible structure of the individual samples and their localization with respect to different plant species and biological structures in the soil. In situations characterized by pronounced spatial patterns, such as many agroforestry systems, it may be useful to analyse the spatial variability of soil porosity with geostatistical methods (see Section 3.6), in which case a large number of measurements (at least 50) have to be taken together with their coordinates in the soil profile and/or on the soil surface. Between the collection of the undisturbed soil samples in the field and the porosity measurements, the samples are stored in the field-moist state at 4°C to limit biological activity.

In a more detailed approach to the analysis of soil structure, the variation of porosity as a function of the sample size within each sampling unit can be studied. This analysis usually reveals that the number of organizational levels within a soil sample increases with the sample size. If the total porosity of a soil sample does not vary with sample size, this

indicates a continuous, massive structure. On the contrary, if the porosity varies with sample size, it is possible to evaluate the different components of the total porosity, each of which is related to an organizational level of the soil structure. At least two such organizational levels can usually be distinguished (Stengel, 1979; Fiès and Bruand, 1990). The assemblage of elementary particles within aggregates determines the textural porosity, and the assemblage of the aggregates determines, together with pores and cavities of biological origin, the structural component of porosity. For this type of analysis, bulk density is measured on samples varying between a few mm^3 and several hundreds of cm^3 in size, using the techniques described in Section 10.2.

Pore size distribution

A common approach to the analysis of soil porosity consists of the determination of pore size distribution, using either mercury injection or water desorption curves on undisturbed samples (Fig. 10.1). Both methods are based on Jurin's law (Eq. 10.2). If the pore space is approximated by a number of capillaries of variable size, but of simple form (cylindrical tubes or fissures with smooth and parallel planes), the capillary pressure P_c, in Pa, of the water is related to the maximum equivalent size of the water-filled pores for each equilibrium state defined by the matric potential of the soil water:

$$P_c = P_0 - P_w = \frac{4\nu \cos \alpha}{d_{eq}} = -h \tag{10.2}$$

where: P_0 = atmospheric pressure; P_w = water pressure (both in Pa); ν = surface tension of water (75×10^{-3} Nm^{-1}) ; α = contact angle of water with the pore walls (0° if the water perfectly moistens the solid particles); d_{eq} = equivalent size of the pore, corresponding to the diameter of a cylindrical capillary (therefore also 'equivalent diameter') or twice the distance between the two walls of a fissure (in m); and h = matric potential of the water (in Pa).

The measurement of pore size distribution allows the total porosity of a soil to be subdivided into size classes of pores which differ in their ecological functions in the soil. Several classifications have been proposed, resulting in considerable confusion in terminology. Some authors divide the pore space into macroporosity (non-capillary pores) and microporosity (capillary pores) according to arbitrarily defined threshold values, such as –60 hPa. These should be adapted to each type of soil and the purpose of the study being undertaken (for the importance of macropores for water infiltration see Sections 7.1 and 11.4). A more comprehensive subdivision of pore space into fissures, transmission pores, storage pores and residual

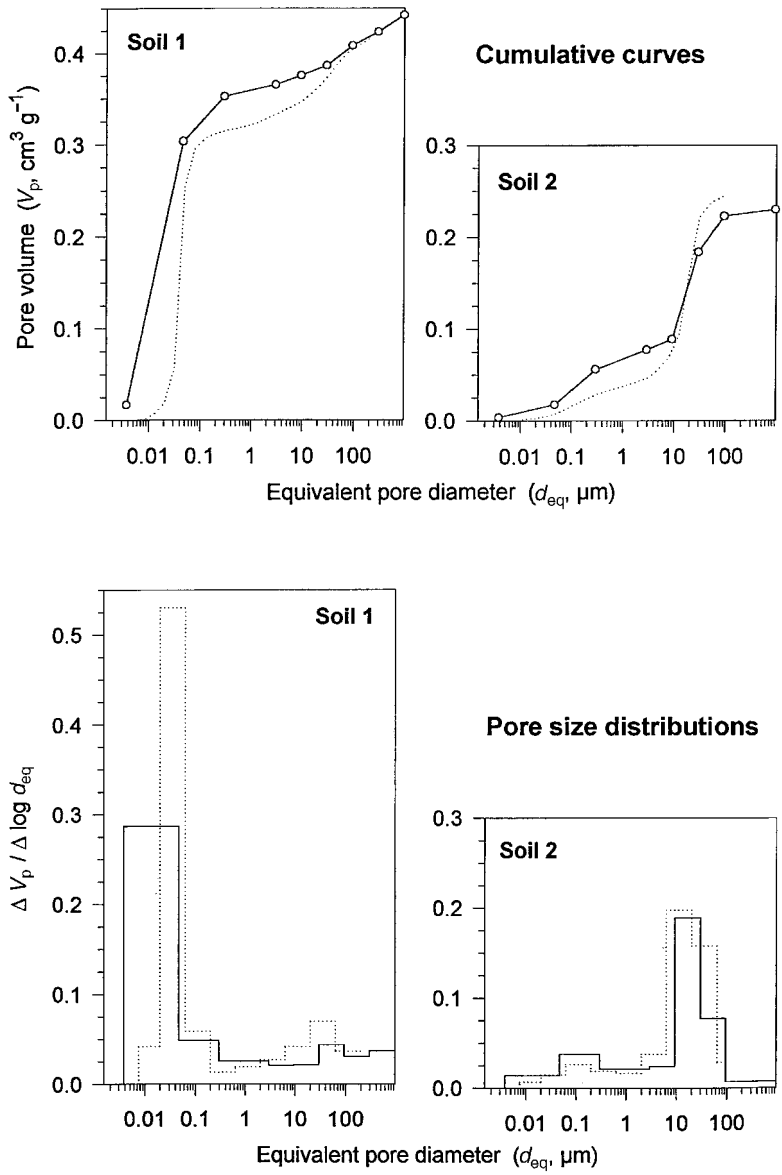


Fig. 10.1. Comparison of pore-size distributions of two Amazonian Oxisols with clayey texture (soil 1, 91% clay) and sandy texture (soil 2, 9% clay) as determined by water desorption (solid lines) and mercury injection (dotted lines). V_p, pore volume; d_{eq}, equivalent pore diameter

pores with limits at equivalent sizes of 500, 50, 5 and 0.5 μm has been proposed by Greenland (1979). An ecologically important division is at 0.2 μm equivalent pore size, corresponding to the conventionally defined permanent wilting point (pF 4.2). Water in smaller pores, within the clay domains, is not available to many plants. The pore space <0.2 μm also corresponds well with the residual water in van Genuchten type models of soil hydraulic properties (see Section 11.4). For a further discussion of pore size classes and their functional properties, see Payne (1988).

Water desorption method

For establishing the water desorption curve, the soil samples are saturated with water on a suction plate or an equivalent device, and are then exposed to progressive desiccation. At each equilibrium state, the mass of the moist sample is measured to calculate its water content. The water volume obtained from the water content equals the volume of the water-filled pores whose maximum equivalent size is given by Jurin's law (Eq. 10.2). Different laboratory techniques are used to vary the matrix potential of the water within the large range between saturation and -10^6 hPa (pF 6) (Tessier and Berrier, 1979; Bruand, 1990). These include suction plates ($h \geq -10^2$ hPa), pressure membranes (-10^1 hPa $\geq h \geq -1.6\ 10^4$ hPa), and desiccators wherein the relative humidity is controlled by a concentrated salt solution ($h \leq -10^5$ hPa).

Mercury injection method

The mercury injection technique is an alternative to the water desorption method for measuring pore size distribution (Lawrence, 1977). Mercury is a liquid which does not moisten the soil. It penetrates a porous, dehydrated and degassed sample if a pressure (P_{Hg}) that is inversely proportional to the size of the penetrated pores is applied:

$$P_{\mathrm{Hg}} = -\frac{4\mu \cos\theta}{d_{\mathrm{eq}}} \tag{10.3}$$

where μ = surface tension of mercury (0.480 $\mathrm{Nm^{-1}}$); θ = contact angle of mercury with the soil particles (141°), d_{eq} as in Eq. 10.2.

Mercury injection is thus physically equivalent to the desorption of water; the mercury permeates the soil in the same way as air permeates a drying soil sample. The analysis of pore size distribution with mercury injection is carried out on small sample volumes (≈ 1 $\mathrm{cm^3}$). The method is appropriate for pores of an equivalent size between 200 μm and 7.5 nm (for a maximum mercury pressure of 10^6 hPa). The technique is rapid and

precise because the injection of the mercury into the sample is continuously recorded, contrary to the desorption of water, which is commonly measured only at distinct suction levels. However, the dehydration of the sample before the measurement provokes a certain shrinking of the soil and thus a modification of the pore space. This shrinking depends on the clay minerals and on the initial structure of the soil. The importance of this modification of the pore space can be evaluated by comparing the results from mercury injection with those from water desorption. In soils with kaolinitic clay mineralogy, the pore size distribution is not seriously altered by the desiccation, but there is nevertheless a slight effect on pore size classes (Fig. 10.1). This effect is not a pure artefact, because the shrinking of the soil is a physical soil characteristic that is interesting to interpret in relation to its structural organization. It should be noted that artificial drying methods such as lyophilization and the critical point method also affect the structure of the soil samples (Murray and Quirk, 1980). It is thus preferable to air-dry and then oven-dry the samples and take the effect of shrinking into consideration in the interpretation of the results.

Pore size analysis with mercury injection can reveal rather small modifications in the structural organization of the soil, as it is sensitive to changes in a wide range of pore sizes, including the pores between clay particles (residual pores), those between larger particles and clay microaggregates (storage pores) and a significant proportion of the structural pores, such as fissures, channels and cavities of biological origin (Fig. 10.2). However, because of the necessary drying of the soil before the measurement, the results can only give indications for a degradation or regeneration of soil structure, but cannot provide a reliable, absolute measure of the evolution of soil porosity. The plant-available water volume which is retained by pores of an equivalent size >0.2 µm is generally underestimated (Table 10.1).

A problem that the water desorption and the mercury injection techniques have in common is that of bottlenecks in the soil pore space. If pores are accessible through narrower bottlenecks, it is the size of these which controls the entry of mercury or the desorption of water (Hillel, 1998). The dimensions of the pores calculated from Eqs 10.2 and 10.3 can thus be underestimated. Comparing mercury injection with image analysis of a microaggregated soil, Colleuille (1993) found that a tenfold underestimation of real pore sizes with porosimetry can occur due to such bottleneck effects.

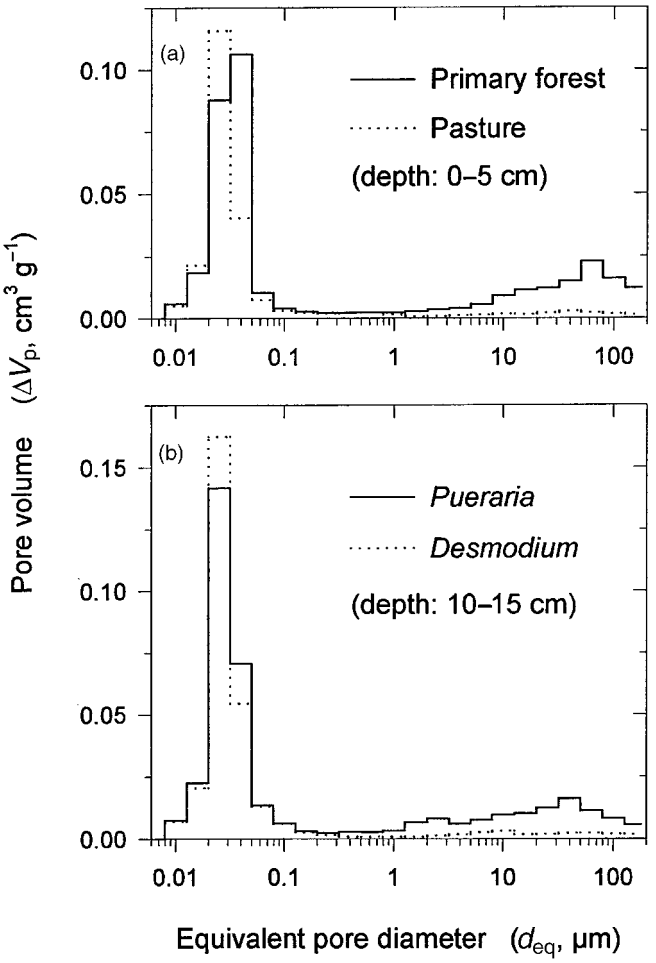


Fig. 10.2. Examples for land-use effects on pore size distribution in clayey Amazonian Oxisols as revealed by the mercury injection technique: (a) effect of cattle trampling in a pasture; (b) effect of two leguminous cover crops (*Pueraria phaseoloides* and *Desmodium ovalifolium*) in an oil palm (*Elaeis guineensis*) plantation on a soil that had previously been compacted by mechanized deforestation (after Grimaldi *et al.*, 1993).

10.5 Measuring the Role of Soil Organic Matter in Aggregate Stability (*G. Schroth*)

As discussed in Section 10.1 and Chapter 4, trees can influence the stability of the aggregate structure of soils by increasing their organic matter

Table 10.1. Comparison of macropore and micropore volumes ($cm^3\ g^{-1}$) as calculated from water desorption and mercury injection data.

Origin of the samples	Depth (cm)	Volume of macropores (200 μm > d_{eq} > 0.2 μm)		Volume of micropores (0.2 μm > d_{eq})	
		Water desorption	Mercury porosimetry	Water desorption	Mercury porosimetry
Primary forest	5–10	0.122	0.078	0.338	0.241
Three-year-old pasture (manual deforestation)	5–10	0.074	0.041	0.349	0.250
Pasture abandoned for 5 years	0–5	0.046	0.025	0.236	0.196
	5–10	0.088	0.042	0.253	0.204
	0–20	0.066	0.055	0.277	0.212
Mechanical compression (10^3 kPa) in the laboratory	–	0.062	0.027	0.334	0.234
Mechanical deforestation and *Desmodium ovalifolium* cover crop	10–15	0.046	0.025	0.371	0.265
Mechanical deforestation and *Pueraria phaseoloides* cover crop	10–15	0.087	0.109	0.359	0.260

content. This role of organic matter is particularly important in sandy soils where clay minerals and iron and aluminium oxides contribute little to the stabilization of the soil structure. Such soils are widespread in West African savannas. Based on the analysis of 495 soil samples from this region, a critical level of soil organic matter, S, was developed, from which the structural stability of the soil and its sensitivity to erosion can be deduced (Pieri, 1989):

$$S = \text{soil organic matter (\%)} / (\text{clay} + \text{silt}) (\%) \times 100 \quad (10.4)$$

A value of $S<5$ indicates structurally degraded horizons with great sensitivity to erosion; $5<S<7$ indicates soils with great risk of structural degradation; $7<S<9$ indicates soils with small risk of structural degradation; and indices >9 indicate soils with sufficient amounts of organic matter to maintain a stable structure.

Of particular importance for the stabilization of soil structure are organic matter constituents with cementing properties such as polysaccharides, which are present in soil as strands or nets of polymers connecting clay particles. Soils rich in oxides and hydroxides of iron and aluminium can have a very stable aggregate structure, in part because strong bonds are formed between polymers and these constituents (Payne, 1988). These polysaccharides can be of plant, microbial or faunal origin. From the five monosaccharides that usually dominate in soils – glucose, galactose, mannose, arabinose and xylose – the second and third are believed to be mainly produced by microbes, and the latter two mainly by plants (Gregorich *et al.*, 1994). Polysaccharides have been found to be more useful for explaining the stability of aggregate structure in arable soils than in grassland and forest soils, possibly because other humus fractions, roots and mycorrhizal fungi are more important for structural stabilization under perennial than under annual vegetation (Payne, 1988; Carter *et al.*, 1994).

Total polysaccharides in soils can be determined by hydrolysis with concentrated sulphuric acid, followed by dilute sulphuric acid. Omitting the first step recovers labile polysaccharides, which are most of the polysaccharides other than cellulose. Detailed procedures are given by Lowe (1993). An increase of dilute acid-extractable carbohydrates was correlated with increased aggregate stability under cover crops in an orchard soil (Roberson *et al.*, 1991). In two Oxisols from the Brazilian *cerrado*, both cellulosic and non-cellulosic polysaccharides were related to macroaggregation (Neufeldt *et al.*, 1999). In other studies, a hot-water (80°C)-extractable carbohydrate fraction was found to be a more sensitive indicator than acid-hydrolysable carbohydrates for changes in soil organic matter quality and aggregation as affected by cropping systems (Haynes and Swift, 1990). The relative merits of dilute acid- and hot-water-extractable polysaccharides as indicators of soil organic matter quality in relation to aggregation are still under discussion (Gregorich *et al.*, 1994).

For ferralitic soils in Queensland, Australia, an active carbon fraction, oxidizable with 33 mM potassium permanganate, was found to be a more sensitive predictor than total carbon for aggregate stability under rain (Bell *et al.*, 1998). Relationships between this active carbon fraction and rainfall infiltration were used to determine critical levels of active carbon to prevent runoff for a given rainfall regime (Bell *et al.*, 1999).

10.6 Soil Micromorphology and Image Analysis
(*M. Grimaldi*)

The study of soil structure is greatly facilitated by the acquisition of images of different magnification with a stereo, optical or electron microscope. A specific terminology is used to describe the arrangement of mineral and organic soil constituents, the morphology of pores, and the origin of aggregates and pores (Brewer, 1964; Stoops and Jongerius, 1975; Bullock *et al.*, 1985; Fitzpatrick, 1990; Ringrose-Voase, 1991). These essentially qualitative descriptions are profitably combined with quantitative image analysis (Hallaire and Curmi, 1994; Ringrose-Voase, 1996), which is based on the concepts of mathematical morphology (Serra, 1982) and allows a full statistical evaluation of soil micromorphological data.

Soil samples with undisturbed structure are collected from profile walls at depths which are chosen in accordance with the horizons and sub-horizons of the pedological profile. The samples are either air-dried, which provokes a certain transformation of their structure, or are kept in the field-moist state at 4°C. In the latter case, they are dehydrated by exchange of water by acetone, followed by impregnation with a polyester resin, to which a fluorescent pigment is added to visualize the pores under ultra-violet light (Hallaire, 1994). After the polymerization of the resin, the blocks are cut and polished to be studied under a stereomicroscope. Thin sections are prepared for analysis with optical or scanning electron microscopy. The use of backscattered electron scanning microscopy results in a more complete characterization of the pore space than that obtained by optical microscopy alone, as it also makes the pores between skeletal grains and clay domains visible, which, in fine-textured soils, are not accessible with other microscopic techniques. Only the very small pores within the clay domains remain invisible even with this method (Fiès and Bruand, 1990). The method was used by Bruand *et al.* (1996) to study the changes of pore organization in the neighbourhood of roots. When biological structures arising from the activity of different soil invertebrates or growing roots can be recognized (Eschenbrenner, 1986), the methods of image analysis are particularly useful for a quantitative characterization of the resulting spatial and temporal changes in the soil structure (Deleporte *et al.*, 1997; Peres *et al.*, 1998; Jegou *et al.*, 1999; Barros *et al.*, 2001).

Image analysis allows us to distinguish and quantify zones of higher and lower density. A large number of parameters related to the image as a whole (image parameters) or to an individual object (object parameters) can be used for the analysis (Ringrose-Voase, 1991). With respect to the pore space, the surface porosity, which is the intersection of the three-dimensional pore space with the image plane, is the image parameter that is the easiest to quantify. Each poroid, or apparently individual pore in the image plane under a given magnification, is characterized by form and size parameters. This allows calculation of the distribution of the surface porosity according to different size and form classes. A frequently used form index is given in the following equation:

$$I = P^2 / (4\pi A) \tag{10.5}$$

where P is the circumference of the poroid and A its area. This allows rounded ($I \leq 5$), elongated ($5 < I \leq 25$) and complex pores ($I > 25$) to be distinguished. To these pore classes, different origins and, to some extent, functions can be ascribed. Elongated pores are mainly fissures that are produced by wetting–drying cycles or by mechanical stress from root growth. Very irregular pores are generally pores formed by the assemblage of aggregates and are often of biological origin. Rounded pores are usually galleries made by invertebrates (e.g. earthworms) or root channels.

A limitation of the use of micromorphological techniques for the analysis of soil structure is that the connectivity of the pore space, which is of fundamental importance for water and gas transport in the soil and thus for drainage and soil aeration, cannot be evaluated reliably due to the two-dimensional nature of the sections. For further information and methodological details see also Fox *et al.* (1993).

Chapter 11
Soil Water

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11.1 Synopsis (*F.L. Sinclair*)

Water is essential for plant growth and, as an inevitable consequence of opening their stomata to enable gaseous exchange during photosynthesis, plants transpire water, which they usually have to take up from the soil. The soil water content also has a pronounced influence on nutrient uptake from the soil as it affects root growth and the transport of nutrients to the root. Furthermore, it influences the availability of oxygen, microbial and faunal activity, leaching of nutrients and agrochemicals into the subsoil, and swelling and shrinking of certain clay soils.

The water content of soil represents a balance between processes that add water to the soil, such as infiltration of rainfall, and processes through which water is lost from the soil, such as plant water use (transpiration), evaporation, runoff and drainage. Typically less than a third of the rainfall input is transpired in crop production systems in dry areas, indicating that there is scope for addition of trees to improve water-use efficiency. This may occur either directly, by capturing more water for plant production, or indirectly, by modification of the microclimate in ways that increase the transpiration efficiency of crops. This book is about soil fertility and so the methods discussed in this chapter focus on aspects of the water balance associated with the soil rather than with plants. In the synopsis, global water supply and demand are discussed, revealing water scarcity as an increasingly widespread agricultural constraint. The components of the water balance are then discussed with particular reference to how the incorporation of trees in agricultural practices may affect the overall

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efficiency of the use of rainfall and impact upon crop productivity. The next two sections explain how soil water content and water potential can be measured. Soil water content refers to how much water there is in soil, while its potential relates to the ease with which it can be extracted. The fourth section discusses measurements of soil hydraulic properties, such as the rate of water infiltration. The final section describes new techniques that use stable isotopes to estimate how much transpired water is being taken up from subsoil, as opposed to surface soil.

Global trends in water resources and tree–crop interactions

Although there is a vast quantity of water on Earth, most of it is saline, frozen or in underground aquifers, so that less than 1% is reasonably accessible fresh water (Wallace and Batchelor, 1997). This accessible fresh water is unevenly distributed and about a third of the world's land surface area is classified as arid or semiarid, indicating that water is a major constraint on agricultural production in these regions (UNEP, 1992). At present, about 7% of the world's population lives in areas where water is scarce, but it is anticipated that this proportion will rise tenfold over the next 50 years, largely because of the increasing water required to grow food for a burgeoning world population, which is growing particularly fast in drier regions (Wallace, 2000). Since about 75% of human use of fresh water is consumed in agriculture, the efficiency with which rainfall is used to produce food, especially where water is scarce, is of fundamental importance to sustaining a global balance between food supply and demand.

The efficiency of use of rainfall in crop production in semiarid areas is low, typically less than 30% for rain-fed cropping and less than 20% for irrigated agriculture (Wallace, 2000). This means that, in principle, integration of trees into agricultural fields could increase overall rainfall-use efficiency by more of the rain being used in plant transpiration because the trees use water not captured by crops, or because they lead to an increase in the amount of water infiltrating into the soil. In addition, trees could modify microclimate, leading to higher transpiration efficiency of crops, resulting in production of more dry matter per unit of water they transpire. But trees may also use a lot of water themselves and although they may access some water not available to crops, either from below the crop rooting zone or at times in the season when crops are not present, they may also compete with crops for water in surface soil. Similarly to tree–crop interactions for nutrients (see Chapter 5), those for water can be described in terms of facilitation (where trees improve crop water use through increased infiltration or transpiration efficiency), complementarity (where trees use water that would not have been used

by the crops) and competition (where trees use water that would otherwise have been used by the crops). As with nutrients, these forms of interaction often occur simultaneously. Research is urgently required to understand the circumstances, in terms of species combinations, the spatial arrangement of tree and crop components, site conditions and management practices, that result in facilitative interactions and complementary water use among trees and crops without incurring a lot of competition. For example, trees in boundary plantings may compete less with crops for soil water than evenly spaced trees in crop fields while retaining much of their beneficial effects on microclimate and infiltration. Some trees are able to grow in swampy and seasonally flooded areas, which are unsuitable for crops, in which case increased landscape water use may be achieved without any negative side effects on the availability of water to crops. Furthermore, there are species differences among trees in their rooting habit and their water use per unit of both root length and leaf area, and in how these respond to management interventions such as pruning (Jones *et al.*, 1998). This makes choice of tree species and their subsequent management vital for achieving benefits of trees in terms of making efficient use of soil water without incurring too much competition for water with associated crops.

A major research requirement is to measure impacts of different tree species and management regimes on soil water depletion and crop yield across a range of agricultural systems and site types. It is especially important to get this information for productive tree species that farmers may be interested in husbanding because they can derive income from them or because the trees contribute to meeting their subsistence needs.

Impacts of trees on water balance

When trees are present in crop fields they have a profound influence on most components of the water balance (Fig. 11.1), which means that soil moisture (θ) will vary spatially in relation to distance from trees because of different inputs and uptake of water caused by their presence. Rainfall is normally the main water input, although in some situations run-on water from upslope areas can be important. Runoff irrigation systems in areas with insufficient rainfall for agriculture are specifically designed to maximize this latter water input (Lövenstein *et al.*, 1991; Lehmann *et al.*, 1999a). Trees affect the amount and spatial distribution of rainfall (P_g) that reaches the soil, with most of the area beneath tree crowns receiving less rainfall than open areas (P_c) because the tree crowns intercept some of the rainfall, which evaporates directly back to the atmosphere (I_t), whereas the rest reaches the soil either as throughfall (P_t) or stemflow (P_s). Wallace (1996) estimated that for sparse tree canopies typical for tropical

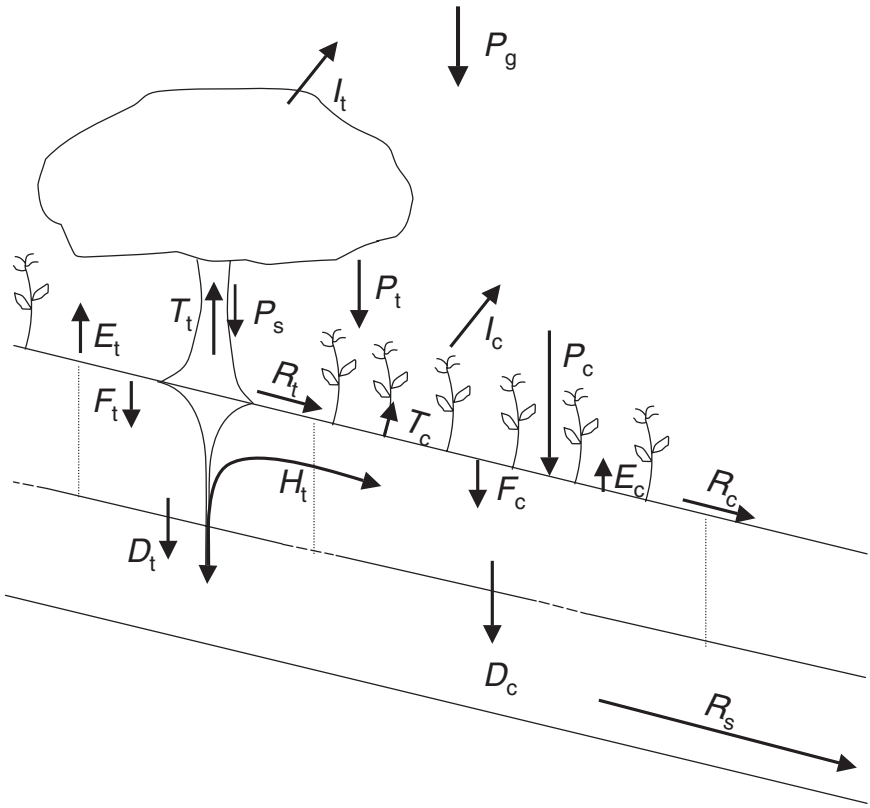


Fig. 11.1. Components of a water balance in a tree–crop system (see text for explanation of symbols). Subscripts $_{c,t}$ refer to crop and tree components, respectively (adapted from Wallace, 1996).

agroforestry systems, interception losses are likely to be in the range of 3–10% of total rainfall, with decreasing relative losses as total rainfall increases. Schroth *et al.* (1999b) measured 6.4% interception in agroforestry plots with different tree crops in Amazonia. Calder (2001) showed that the larger drop sizes in tropical convective storms reduce interception losses compared with trees in temperate climates. The division of rainwater into throughfall and stemflow during crown passage influences the spatial pattern of water inputs into the soil, and consequently soil water content and drainage. Certain tropical trees such as palms may concentrate considerable amounts of rainwater as stemflow in their proximity, thereby possibly reducing its availability to associated crops. The water input into a 2 m^2 area around the stems of peach palms (*Bactris gasipaes*) in an Amazonian agroforestry system was 152% of open-area rainfall (Schroth *et al.*, 1999b).

Another major effect of the tree crowns is to intercept radiation and so reduce the energy reaching the soil or crop beneath. This can substantially reduce evaporation of water from bare soil under trees (E_t), which is important because more than 30% of total rainfall is typically lost through evaporation from soil in rain-fed cropping systems in the semiarid tropics (Wallace, 2000). The presence of 50% tree cover in an agroforestry system in semiarid Kenya was found to reduce cumulative soil evaporation over a season from about 55% of the rainfall input to 39% (Wallace and Batchelor, 1997; Wallace *et al.*, 1999). Soil evaporation is also reduced by tree litter and mulch (Wallace, 1996).

Trees may also enhance infiltration (F_t) over that occurring on areas with crops alone (F_c) by protecting the soil surface from rain splash with their litter and mulch and increasing soil faunal activity, thereby reducing runoff (R_t). Where trees are planted on the contours, runoff water from cropped surfaces may infiltrate under the trees, where infiltration rates may be higher than under the crops (Kiepe, 1995; see Chapter 17). Drainage may also be lower in the vicinity of trees (D_t) because water is taken up from more of the vertical extent of the soil profile than in areas where only crops are present and this water is subsequently transpired (T_t). In dry areas, trees may be able to access water from several tens of metres depth (Leakey *et al.*, 1999). Water uptake by trees may also reduce subsurface lateral flow of water (R_s). Lateral drainage occurs on slopes and may be especially important where sandy topsoils overlie clayey subsoil horizons of lower permeability, a situation commonly encountered in some tropical regions (Chauvel *et al.*, 1987). Runoff and drainage typically account for 40–50% of rainfall input in semiarid rain-fed cropping systems (Wallace, 2000). Tree root systems may also redistribute water vertically in the soil profile by absorbing water from wet soil at one depth and exuding it in dry soil either higher or lower in the profile (H_t). This was first observed in the upward direction, where shrubs took up water from moist soil at depth and exuded it in dry soil near the surface, enabling nutrient uptake by shrubs and growth of associated herbaceous plants, and was termed hydraulic lift (Caldwell and Richards, 1989). More recently the process has been found to operate in reverse with water travelling down roots from wet surface soil to be exuded at depth, facilitating root growth through dry soil to reach water and nutrients that are available at depth in some arid ecosystems (M. Smith *et al.*, 1997; Schulze *et al.*, 1998).

Over an annual cycle the trees will also take up and transpire water at times when the crop is not present or when it has only a small leaf area and root length. The extent of the seasonal complementarity of water use will depend on the rainfall distribution at the site and the phenology of leaf development and retention by the trees. The reverse phenology of *Faidherbia albida*, a tree species of African savannas which loses its leaves during the wet season, is likely to lead to temporal complementarity of

water use with annual crops. Roupsard *et al.* (1999), monitoring water use of adult *F. albida* trees in a Sudanese parkland, found that the tree roots accessed a water table at about 7 m depth and so were able to be productive during the dry season, with the fraction of annual rainfall used by trees remaining below 5%, indicating little competition with annual crops grown in the wet season. Similar effects can be achieved through strategic crown pruning of trees during dry spells or phases with high crop water requirements (Smith *et al.*, 1998). Surprisingly little is known about the phenology of many trees with agroforestry potential, so measuring seasonal water extraction from soil by trees with different phenology, to find species that will exhibit complementarity with different cropping patterns under various rainfall regimes, is a key area for research.

Transpiration efficiency

In addition to the impacts on the amount of water captured for transpiration, trees may also increase the transpiration efficiency of crops by reducing the saturation deficit of the air beneath their crowns. Overhead tree shade generally reduces radiation receipt and air temperature, and increases humidity experienced by the understorey. The presence of tree crowns may also reduce wind velocity (Green *et al.*, 1995) and so reduce mixing of air, maintaining a low saturation deficit over the crop. This means that less water will be transpired per unit of dry matter produced by the crop because, when stomata open to allow gaseous exchange in photosynthesis, less water evaporates from leaves (Wallace and Verhoef, 2000). Effects of shelterbelts may be more complex because the shelter is provided laterally so that wind speed is reduced but there is no overhead shade to reduce radiation receipt. This can lead to higher temperatures and saturation deficits at leaf surfaces in the lee of the windbreak and hence greater water loss per unit of dry matter accumulation (Brenner *et al.*, 1995). Shading could also improve transpiration efficiency by reducing heat stress that unshaded crops frequently experience in areas of high incident radiation and temperature (Ong *et al.*, 2000).

The measurement of the plant and atmospheric components of the water balance in tree–crop systems has been discussed and described by Wallace (1996). In recent years major advances have been made in direct measurement of water uptake by plants using heat as a tracer to measure sap flow rate. The various techniques for measuring sap flow in stems of trees and crops have been reviewed by Smith and Allen (1996) and their application to tree and crop roots by Fernandez *et al.* (2000). Another recent development of particular relevance to agroforestry, the use of stable isotopes to estimate the proportion of plant water uptake from

subsoil as opposed to surface soil, is discussed in Section 11.5. Measurement methods for soil water content, water potential and soil hydraulic properties are discussed in the following sections.

11.2 Methods for Soil Water Content (*W.G. Teixeira, B. Huwe*)

Measurements of soil moisture express the amount of water in a given mass or volume of soil. The water content is usually reported in cm cm^{-3} or in g g^{-1} relative to the dry soil. Soil water content (and water potential) are highly variable in both space and time, changing markedly after each rainfall event. Addition of trees to cropping systems exacerbates this variability. Ideally, therefore, it is desirable to record soil water data automatically at short time intervals for a large number of points in horizontal and vertical positions in the soil. In large experiments with numerous measurement points the cost of equipment to make automatic measurements over the whole experiment may be prohibitively high. A useful compromise between minimizing cost and maximizing temporal and spatial resolution is to conduct infrequent, manual readings at many measurement points within a study site combined with frequent, automated readings at a few points.

Gravimetric method

The gravimetric measurement of soil water content involves the weighing of collected soil samples before and after drying at 105°C to constant weight. The principle of the method is simple but the sampling and laboratory procedures are labour intensive. The method is destructive, which makes it impossible to resample the same site and automate the acquisition of data. The measurement can be carried out on either disturbed soil collected with a soil auger or undisturbed soil collected with steel cylinders of known volume. The deviations normally found among data from disturbed and undisturbed samples are related to the fact that the mass-based values from disturbed samples are recalculated to volumetric values by means of the measured bulk density, thus introducing a new source of error. For gravimetric methods, normally taken as the 'correct' value of soil moisture, the principal sources of inaccuracy are (Gardner, 1986):

- an inappropriate sampling scheme;
- an inadequate number and volume of samples;
- uncertainties in the equilibrium time when drying the sample (if repeated weighing of the sample is not conducted);

- the presence of colloidal material that retains water even when exposed to high temperatures;
- the presence of organic material that can oxidize or volatilize, leading to specific problems in some organic soils;
- difficulties in maintaining a constant temperature in the oven; and
- the precision of the balance used to weigh samples.

Soil samples can also be dried by microwaves but this method may not give reliable results for soils containing significant amounts of mica, gypsum, halloysite, montmorillonite or other hydrated materials common in organic soils (Liu and Evett, 1990).

The direct gravimetric method is the most commonly used method in soil water studies in tropical agroforestry. The effect of hedgerow intercropping on soil moisture determined by gravimetric methods has been investigated in numerous studies in the humid, subhumid (Lawson and Kang, 1990) and semiarid tropics (Lal, 1989; Rao *et al.*, 1991), focusing on the balance between beneficial and competitive effects between trees and crops. For example, detailed measurements of the soil water profile at Machakos, Kenya, substantiated the importance of competition of hedgerows for water in semiarid environments, which often leads to reduced crop yields despite beneficial windbreak effects of the trees (Lal, 1989; Rao *et al.*, 1998). Sequential coring and gravimetric determination of soil water content were used by Jones *et al.* (1998) to show differences among tree species in water uptake from surface soil, differences in how species responded to shoot pruning and hence their competitiveness with crops and the extent to which this could be controlled by management interventions. Biweekly gravimetric determination of soil moisture was also used in a nutrient leaching model for an agroforestry system with cocoa and shade trees (either *Cordia alliodora* or *Erythrina poeppigiana*) in Costa Rica (Imbach *et al.*, 1989).

Neutron scattering

The neutron-scattering method is a non-destructive procedure developed in the 1950s and still in use in many research centres. It uses the principle of neutron thermalization. High-energy neutrons that are emitted from a radioactive substance, such as ^{241}americium-beryllium, slow down and scatter by elastic collisions with hydrogen nuclei. The rebounding neutrons are measured with a detector (Greacen, 1981; Gardner, 1986). The sphere of influence of the emitted neutrons is roughly spherical with a radius of about 10 cm in wet soil and 25 cm in dry soil (Hillel, 1998). When measurements are carried out close to the soil surface, the sphere may extend into the air, making measurements in the topsoil both unreliable

and a safety hazard. The measurement device is inserted into the soil through thin-walled aluminium access tubes, which are installed in holes in the soil created with a soil auger. Because of interactions with other atoms in the soil, such as cadmium, lithium, boron and chlorine, and the fact that hydrogen is present in soil organic matter and as structural water in some soil mineral components, extensive calibration against gravimetric water contents may be required (Greacen, 1981; Gardner, 1986; Grimaldi *et al.*, 1994). The calibration equation is normally linear, but to determine the slope accurately it is essential to sample a sufficiently wide range of dry and wet points (Greacen, 1981). Measurement bias resulting from instrument drift is partially avoided by the normalization of the observed count rate by the count rate in pure water, which is strongly recommended (Williams and Sinclair, 1981). In agroforestry research, changes in soil organic matter content and thus hydrogen concentration of the soil provided by sources other than water may necessitate periodic recalibration of the method.

An advantage of neutron probes is independence of the measurements from temperature and pressure (Kutílek and Nielsen, 1994). The major disadvantages are radiation hazard and the infeasibility of making measurements near the soil surface. The radioactive device must be handled with extreme caution and stored properly and cannot be installed in the field for automated data collection. Safety regulations require training of the users and government licences for the ownership, transport and use of a radioactive source.

Examples for the use of the neutron-scattering method in agroforestry research include Singh *et al.* (1989) and Govindarajan *et al.* (1996) in studies of tree–crop interactions in hedgerow intercropping in India and Kenya, respectively, and Eastham *et al.* (1994) in studies of water-use efficiency of fodder trees in silvopastoral systems in Australia.

Time domain reflectometry (TDR)

This method is increasingly used as a non-invasive technique for the determination of the volumetric water content of soils. It is based on the determination of the dielectric constant (ε) of the soil through the measurement of the propagation velocity of electromagnetic waves, using the large difference of ε between water (≈ 81), air (≈ 1), mineral constituents of soil (3–5), and frozen or bound water (≈ 3.2). The dielectric constant is measured with probes that are installed in the soil, down to a depth of several metres, if required, and that are either permanently connected to a datalogger or are temporarily connected to a mobile device. Certain devices allow instantaneous measurements of topsoil moisture with minimum soil disturbance with mobile probes that are inserted vertically

into the soil when measurements are to be made. It is also possible to use the TDR technique with access tubes as in the neutron-scattering method.

TDR offers the possibility of continuously monitoring changes in soil water content, even at remote sites. The technique involves a relatively large initial investment for the acquisition of the equipment, but it can be easily installed in measuring networks with automated data collection and, for large numbers of measurement points, it can be less expensive than neutron probes, although it is generally more expensive than frequency domain methods (see below). The role of the TDR technique in agroforestry research is likely to increase in the future.

It was initially believed that a single universal equation could be found relating soil water content (θ) to ε. However, subsequent work showed that various factors may influence the measurement of ε, so that site-specific calibrations may be necessary for improving the accuracy of θ estimates, especially in clayey soils, soils with low bulk density (Roth *et al.*, 1992; Dirksen and Dasberg, 1993; Malicki *et al.*, 1996) and soils with high organic matter content (Herkelrath *et al.*, 1991; Roth *et al.*, 1992). The empirical calibration equations generated in temperate soils frequently give inadequate accuracy in tropical soils (Dirksen and Dasberg, 1993; Weitz *et al.*, 1997). For example, in a central Amazonian Oxisol with about 70% clay content and a bulk density around 0.9 Mg m^{-3}, use of the built-in, temperate-zone calibration of a TDR device led to errors of 0.10 m^3 m^{-3} (Teixeira, 2001). The temperature effect on determination of ε is reported to be linear (Pepin *et al.*, 1995; Persson and Berndtsson, 1998) and should be corrected where large temperature fluctuations occur, especially near the soil surface. Under saline conditions or in the presence of large amounts of fertilizer, which can interfere in ε measurements (Dalton, 1992; Kim *et al.*, 1998), more accurate measurements can be obtained with coated buriable waveguides. In some tropical soils, high iron content and the presence of magnetic minerals can likewise interfere in ε determinations (Roth *et al.*, 1992; Robinson *et al.*, 1994). Calibration procedures using empirical (Topp *et al.*, 1980; Malicki *et al.*, 1996) or physically based (Roth *et al.*, 1990; Weitz *et al.*, 1997) relationships between ε and volumetric soil moisture can be found in the literature.

When installing the probes in the field, air pockets, stones and the channelling of water along the probes need to be avoided. In agroforestry research, care should also be taken to avoid bias due to small-scale variations in soil properties caused by vegetation or management actions that may affect the soil water content. Soil temperature and bulk density may vary over short distances in the field because of differences in leaf litter, root density or tillage, and their influence on the TDR calibration should be checked at the beginning of a measurement programme.

Because of the recent development of the TDR technique, few agroforestry applications have so far been reported. Yunusa *et al.* (1995)

used the method together with neutron scattering to analyse plant water use and deep drainage in a silvopastoral experiment with pines in New Zealand. V.L. Grime and F.L. Sinclair (unpublished) used the technique together with gravimetric methods in a large agroforestry experiment with dispersed trees and sorghum on vertisolic soils on the edge of the Sahel in northern Nigeria, while Wallace (1996) has used TDR in semiarid conditions in Kenya and Teixeira (2001) in tree–crop agroforestry in central Amazonia.

Frequency domain reflectometry (FDR) or capacitance method

This method is based on the measurement of the resonance frequency when high-frequency electrical waves are created around a sensor (Dean *et al.*, 1987). The sensor basically consists of a pair of electrodes that form a capacitor, with the soil acting as the dielectric medium. Alteration in soil water content is detected by changes in the operating frequency. Recent systems use probes permanently buried in the soil or mobile probes inserted through access tubes as in neutron-scattering measurements.

Advantages of the capacitance methods are the possibility of connection to conventional data loggers and their relatively low cost. The method requires that probes are calibrated for each soil. Separate calibrations are necessary for positions or horizons differing in bulk density. Many of the advantages of TDR are also valid for FDR including rapid, repeatable measurements and near-surface measurements. The disadvantages are greater sensitivity to salinity and to air gaps or cracks in the soil around the electrodes (Evett and Steiner, 1995; Paltineanu and Starr, 1997). Careful installation of access tubes is, therefore, essential.

Other methods

These include gamma ray attenuation (Gardner, 1986) and remote sensing by ground-penetrating radar (Weiler *et al.*, 1998). It is also possible to estimate soil moisture indirectly from soil water potential through parametric models (Campbell, 1974; van Genuchten, 1980). This approach has been used in a hedgerow intercropping experiment with *Leucaena leucocephala* and *Flemingia macrophylla* in Zambia (Chirwa *et al.*, 1994b). It has to be used extremely carefully, as hysteresis can lead to incorrect estimates of soil water content (see Section 11.3).

Table 11.1 summarizes the methods for evaluating soil moisture content.

Table 11.1. Methods for evaluating soil moisture content.

Method	Principle	Range ($m^3\ m^{-3}$)	Accuracy ($m^3\ m^{-3}$)	Collection of data	Requirements/limitations
Gravimetric	Weighing of soil samples before and after oven drying	0–1	±0.001–0.05	Manual, destructive sampling	Accuracy of balance ±0.01
Neutron scattering	Determination of rebounding thermalized neutrons	0–1	±0.01	Electrical, manual readings	Calibration is necessary; recalibration could be necessary with changes in soil organic matter
Time domain reflectometry	Determination of the velocity of propagation of electromagnetic waves in dielectric medium	~0.1–0.7	±0.01–0.05 in mineral soils; ±0.07–0.18 in organic soils	Electrical, manual or automatic readings	Specific calibration is necessary, especially in organic and clayey soils; cable and probe rods should be periodically checked
Frequency domain reflectometry	Determination of resonant frequency in dielectric medium	~0.1–0.5	±0.05	Electrical, manual or automatic readings	Sensitivity is concentrated near the probe; recalibration is recommended if the sensor is changed during the experiment

11.3 Methods for Soil Water Potential (*W.G. Teixeira, B. Huwe*)

The soil water potential can be interpreted as the work (per unit mass, weight or volume) necessary to remove water held in the soil under the influence of matric (or capillary), osmotic and gravitational forces. It is commonly measured in centimetres of water column or its decadic logarithm, the pF value. Information on the soil water potential is essential in studies of water transport, including water uptake by plants. The relationship between the matric potential (or pressure head) and water content of a soil is a function of its pore size distribution (Fig. 11.2; see also Section 10.4). At a given water potential, clayey soils normally have a greater water content than sandy soils, but they also hold a larger part of the water in very fine pores, where the water is not available to plants. The relationship between soil water content and water potential is also influenced by soil structure (Fig. 11.2). The available water capacity of a

Fig. 11.2. Typical soil water retention curves of a sandy soil, a clayey soil and a well-structured, clayey Oxisol. For the latter soil, note that the pattern of water release indicates a bimodal distribution of soil pores. The vertical lines indicate field capacity (pF 1.8) and the permanent wilting point (pF 4.2).

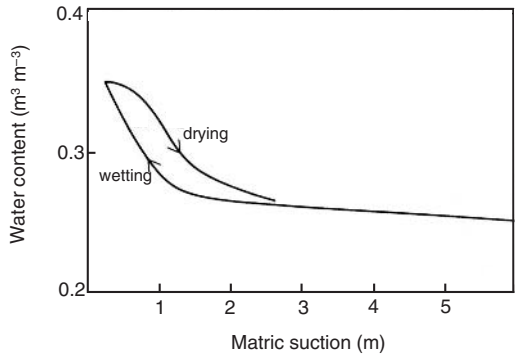


Fig. 11.3. Hysteresis in the relationship between water content and potential during the wetting and drying of a clay loam soil in the field (adapted from Marshall and Holmes, 1979).

soil is defined as the difference between field capacity and permanent wilting point. The field capacity is the amount of water held by the soil against gravity, after excess water has drained away. The exact value is a matter of definition and is conventionally measured at a matric suction somewhere between 5 and 33 kPa. The permanent wilting point characterizes the water content at which all water is held in very fine pores from which it cannot be extracted by plant roots (see Section 10.4). It is conventionally measured at a matric suction of 1.5 MPa (pF 4.2), although the true value differs between plant species (Gregory, 1988).

The relationship between water content and water potential of a soil displays hysteresis, which means that soil that has reached a certain water potential by wetting will have a lower water content than soil that has reached the same water potential by drying (Fig. 11.3). A detailed discussion of this phenomenon is given by Hillel (1998) and Iwata *et al.* (1995).

There are numerous techniques to measure the soil water potential. Generally, these involve the measurement of some property of the water in a reference medium that is in thermodynamic equilibrium with the liquid or gas phase of the water in soil.

Tensiometers

These are the most common instruments for measuring the soil water potential in the field. They consist basically of a porous cup in the soil connected to a water-filled tube that transmits the soil water potential to a measurement device such as a manometer or electrical pressure transducer. Portable pressure transducers (tensiometers) can reduce costs

when a large number of instruments are needed. However, they are sensitive to temperature fluctuations and may require correction. The air-entry capacity of the commonly used porous cups restricts the measurement range of tensiometers to suctions less than about –85 kPa at sea level with an accuracy of approximately 0.1 kPa (Cassel and Klute, 1986). At higher altitudes the reduced atmospheric pressure causes further reductions in the operating range. Because the absolute pressure inside the tensiometer includes the hydraulic head of the water column in the tube, the maximum depth to which a conventional tensiometer can be usefully operated below the soil surface is about 4 m (Livingston, 1993). At lower depths, tensiometers can be operated from soil pits.

Tensiometers are often installed in groups at different depths. Before installation, each tensiometer should be calibrated as shown by Livingston (1993) and Cassel and Klute (1986). Intimate hydraulic contact between the ceramic cup and the surrounding soil is essential for reliable measurements. In most situations the tensiometer can be installed in a tight access hole. Sometimes installation at an angle may be helpful to prevent water flow along the tubes, but the corresponding reduction of the pressure head in the tube needs to be considered. In experiments of long duration with trees or perennial crops, preferential root growth around the ceramic cups can lead to unrepresentative readings requiring periodic reinstallation of the tensiometers. Frequent maintenance is required under hot and dry conditions, especially in sandy soils or shrinking clays. As soils dry out, air bubbles appear in the tensiometer, which do not obviate the measurement but reduce its sensitivity. They can be removed by applying suction or refilling the tensiometers with degassed water. Tensiometers are also susceptible to errors induced by temperature fluctuations and, therefore, the above-ground parts should be shielded from direct exposure to the sun. In hot regions it is recommended that readings are taken from tensiometers in the morning and always at the same hour. Where freezing temperatures occur, tensiometers have to be removed or emptied during the cold season.

In the field, the variance of tensiometer readings often increases with the mean tension (Greminger *et al.*, 1985; Saddiq *et al.*, 1985; Hendrickx *et al.*, 1990), and this may cause problems with some statistical tests and require transformation of data prior to analysis. The variance of tensiometer readings can be reduced by increasing the cup size (Hendrickx *et al.*, 1994).

The main problem with tensiometers is that they operate only in the relatively wet range of soil water potential, so their use in combination with other methods may be necessary. A recent capacitive technique allows measurements of soil water potential in the range 0–1000 kPa (Equitensiometer, Delta T, Cambridge), although at reduced precision. Other field methods for the dry range of soil water tension include heat

dissipation (Phene *et al.*, 1992; Reece, 1996) and soil psychrometry (Rawlins and Campbell, 1986).

Tensiometry has been used in agroforestry research to determine temporal and spatial patterns of soil water potential and indirectly the activity of plant roots. Examples are the measurement of moisture depletion patterns under hedgerow intercropping with different tree species in Nigeria (Kang *et al.*, 1985; Lawson and Kang, 1990), effects of boundary plantings on water availability in an adjacent crop field in Togo (Schroth *et al.*, 1995a), interference between neighbouring plots through lateral tree roots in Nigeria (Hauser and Gichuru, 1994) and tree–crop interactions in runoff agroforestry in semiarid northern Kenya (in combination with gypsum blocks) (Lehmann *et al.*, 1999a).

Gypsum block sensors

These sensors consist of two electrodes embedded in a porous block of gypsum ($CaSO_4$). When installed in the soil, the electrical resistance (Ω) measured between the electrodes is determined by the water content of the blocks at equilibrium with the moisture potential of the surrounding soil. The soil water potential can be inferred with proper calibrations. Gypsum blocks are probably the cheapest sensors of soil water potential; they can be easily installed and connected to a data logger.

For calibration, blocks are equilibrated with the respective soil at different water potentials, normally using a pressure plate. Individual calibrations are necessary for each block and for each soil horizon if these differ significantly. The calibration equations normally show non-linear inverse relationships between resistance and soil moisture potential (Campbell and Gee, 1986; Livingston, 1993). Temperature correction is necessary when the calibration temperature differs from that in the field; correction factors are normally supplied by the manufacturer. The rather high solubility of the gypsum used in the blocks leads to changes of the calibration parameters with time, especially in acid or alkaline soils and where the water table is frequently at high levels. Likewise, changes in the properties of the soil matrix may require periodic recalibration in long-term experiments.

Although gypsum blocks can measure soil water potential in the range between –50 and –1500 kPa, this internal range may vary according to technical characteristics of fabrication. With calibration and temperature correction, the precision of soil moisture potential measurements is likely to be around $\pm$0.1–0.5 MPa (Livingston, 1993). The low sensitivity of gypsum blocks in the dry range and restricted contact between the block and the surrounding soil make them unsuitable for use in sandy soils or shrinking clays (Gardner, 1986). The presence of salts such as fertilizer

leads to an overestimation of soil wetness (Young and Warkentin, 1975).

Agroforestry applications of gypsum blocks include Huxley *et al.* (1994), who monitored the position of wetting and drying fronts in the soil profile in a tree–crop interface experiment with uncalibrated blocks in Machakos, Kenya, and Lehmann *et al.* (1999a) in northern Kenya.

Granular matrix sensor

The granular matrix sensor (Watermark Soil Moisture Sensor, Irrometer Co., Riverside) operates with the same electrical resistance principle as gypsum blocks. It also contains a wafer of gypsum embedded in a granular matrix to minimize salinity effects (Eldredge *et al.*, 1993). The granular matrix sensors reduce some of the problems encountered with gypsum blocks. Of particular significance is their greater stability and thus longer lifetime in the soil. They exhibit sensitivity to soil water potential over a range from 0 to –200 kPa and can, therefore, be used in drier soils than tensiometers and in wetter soils than gypsum blocks. This makes them adaptable to a wider range of soil textures than gypsum blocks. However, granular matrix sensors also require proper calibration and temperature correction.

Other methods of measuring soil water potential are described in Hillel (1998) and Klute (1986).

11.4 Methods for Soil Hydraulic Properties (*W.G. Teixeira, B. Huwe*)

The mobility of water in the soil is determined by soil hydraulic properties, particularly its saturated and unsaturated conductivity, which are functions of the soil water content or water potential (Fig. 11.4). These soil characteristics influence processes such as water infiltration (and thus runoff and erosion), its transport to plant roots, and leaching of nutrients and pesticides. They are thus an essential input in models of water and nutrient cycling. Hydraulic properties are a function of the number, volume, morphology, connectivity and stability of soil pores. They are affected by soil characteristics that influence the cohesive forces between soil constituents, such as pH, cation exchange capacity, iron and aluminium oxides, and organic matter (Hillel, 1998). Hydraulic properties are spatially and temporally variable and are easily modified by factors such as tillage, fertilization, use of mulch and cover crops, root growth, and faunal and microbial activity.

In this section, some field methods for evaluating soil hydraulic properties above the water table are described. Laboratory techniques and

Fig. 11.4. Relationship between hydraulic conductivity (*K*) and matric suction evaluated with a tension infiltrometer from the soil surface near two tree crops on a central Amazonian Oxisol.

methods below the water table are given by Stephens (1996), Carter (1993) and Klute (1986). One of the most important considerations in measuring hydraulic properties in the field is their large spatial variability. The presence of macropores or soil cracks commonly found in certain tropical soils further increases this variability. For comparisons between different experimental treatments, it is often better to have numerous measurement points with smaller precision than just a few highly accurate data points.

Infiltrability and saturated hydraulic conductivity

Infiltration rate refers to the vertical entry of freely available water into a soil surface. It should not be confused with hydraulic conductivity or permeability, which is a measure of the ability of a soil to transmit water in a three-dimensional system. In infiltration experiments, the infiltration rate tends to be numerically equal to the saturated hydraulic conductivity if the hydraulic gradient of the soil is unity. This condition is frequently approximated for homogeneous and isotropic soils (Black *et al.*, 1969; Libardi *et al.*, 1980). If incorrect, the assumption of a unit gradient can be a source of error in the calculation of the hydraulic conductivity (Ahuja *et al.*, 1988; Reynolds and Zebchuk, 1996). Common causes of divergence from the unity gradient include entrapped air within the narrow pore space when an initially dry soil is wetted, and the occurrence of horizontal flows (Collis-George, 1977; Mbagwu, 1995). Nevertheless, for many

practical problems of large-scale significance, the mean infiltration path over a sufficiently large area is approximately one-dimensional in the vertical direction, especially in relatively homogeneous soils (Stephens, 1996).

In tropical climates, the exposure of the soil surface frequently leads to the formation of a superficial soil crust. To verify if such a thin, superficial layer of low permeability controls the infiltration rate, measurements should be made first under natural surface conditions and then after the surface layer has been carefully removed. Worms tend to move upwards and crawl out of the soil when the surface is covered with water (Bouwer, 1986). The resulting open wormholes can greatly increase infiltration rates, particularly if the test is carried out over a long time. Prewetting the site before the measurements can alleviate this problem, which is frequently encountered in the tropics. The temperature and chemical composition of the water used for infiltration measurements should be the same as those of the soil water to avoid dissolution of soil air in the infiltrating water (Bouwer, 1986) and changes of the flocculation status of the clay particles (Hillel, 1998). If possible, rainwater from the site should be used to avoid such problems. The conditions of the measurements such as water quality, temperature, soil moisture and surface conditions, as well as the exact procedure of the prewetting and infiltration, should be recorded so that they can be duplicated in subsequent measurements.

Double-ring infiltrometer

The double-ring infiltrometer consists of two concentric metal cylinders that are pushed or driven a small distance into the soil. The cylinders are filled with water, and the infiltration rate is measured until it reaches a constant value in the inner ring (Bouwer, 1986). The simplicity and low cost of the method are its main advantages.

As the head of water ponded on the soil surface affects the infiltration rate, a constant water level should be maintained in the cylinder with a float valve or a mariotte siphon arrangement. To prevent leakage between the cylinders, equal water levels must be maintained in both cylinders. A multiple automated system can be constructed to allow simultaneous measurements with several devices (Matula and Dierksen, 1989; Maheshwari, 1996).

The use of a double ring, with measurements confined to the inner ring, minimizes errors due to non-vertical flow at the edge of the cylinder. However, in many tropical soils the flow below the infiltrometer is often not straight downward but diverges laterally. Hence the infiltration rate as measured in the infiltrometer will be greater than the true infiltration rate for vertical flow. To reduce this source of error, the outer cylinder

should be as large as possible, even though big rings are cumbersome and require a large volume of water. To reduce the lateral divergence of the flux, the soil sites to be tested should be artificially prewetted, or the measurement should be carried out after rain has fallen. If the measurement cannot be carried out on the same day as wetting the soil, the site should be covered with a plastic sheet or plant residues to prevent surface drying. A restricting layer deep in the profile can cause overestimation of the infiltration rate, because lateral flow above this layer may be greater under measurement conditions than when the entire soil surface is inundated and all water has to move straight down through the soil and the restricting layer (Bouwer, 1986; Kutílek and Nielsen, 1994). In this situation it is preferable to use another technique such as the borehole permeameter (see below).

In addition to lateral flow of infiltrating water, several other factors can affect the results from double-ring measurements. The soil compaction caused by the insertion of the cylinders can lead to a reduction of the true infiltration rate. On the other hand, the measured infiltration rate will be overestimated if a surface crust or other restricting layer at or near the surface is disrupted by the installation of the infiltrometer, or if there is imperfect contact between the restricting layer and the inside cylinder wall (Bouwer, 1986).

The commonly used equations to describe infiltration results are the Philip's two-parameter equation, Green–Ampt equations and the empirical equations of Kostiakov and Horton. The applicability and utility of these different equations are discussed by Hillel (1998), Jury *et al.* (1991), Kutílek and Nielsen (1994) and Libardi (1995). Recently, methods to calculate infiltration from a single-ring infiltrometer using scaling approaches have been published (Wu and Pan, 1997; Wu *et al.*, 1999).

An extension of the double-ring method that allows testing of the assumptions of the infiltration measurement and subsequent determination of unsaturated conductivity has been developed by Ahuja *et al.* (1976, 1980). The double-ring infiltrometer is used in combination with two multiple-depth tensiometers installed in the inner and outer rings (simple tensiometers would require many installation holes within a small area). The tensiometers are used to ensure that a steady state is reached during the infiltration measurement, to estimate the lateral flow components, to determine vertical infiltration rates and to estimate the saturated hydraulic conductivities of the different soil horizons. After the steady-state parameters are obtained, a plastic sheet is placed on the soil to prevent evaporation, and the soil water tensions are periodically recorded during the subsequent drainage period. From these, the unsaturated hydraulic conductivity as a function of soil water tension $K(\psi)$ is obtained. The unsaturated hydraulic conductivity can also be measured as a function of soil water content $K(\theta)$ with multiple TDR probes. This

can be advantageous because the hysteresis of $K(\theta)$ is less than that of $K(\psi)$ (Mualem, 1986). The use of neutron probes or TDR in access tubes to measure the soil water content is also possible, although the installation of the wider access tubes in the small ring area can disturb the soil.

Rainfall simulators or sprinkler infiltrometers

These are arrangements of droppers or nozzles supplying the soil surface with a uniform inflow of water such that the flux density at the soil surface is kept at a constant level (Peterson and Bubenzer, 1986; Kutílek and Nielsen, 1994). The principal advantage in comparison with double-ring infiltrometers is a better simulation of the infiltration process under natural rainfall conditions (Bouwer, 1986; Wallace, 1996). The relatively large sample area of these infiltrometers should increase the representativeness of the measurements. Small systems are available for small-scale applications in agroforestry plots (Asseline and Valentin, 1978; Mathieu and Pieltain, 1998).

Borehole infiltrometer, well permeameter or Guelph permeameter

This is a subsurface technique consisting of a mariotte flask that is lowered into a well or borehole augered into unsaturated soil to the desired depth of measurement. The mariotte flask maintains a constant depth of water in the hole as a means of measuring the rate of water flow into the surrounding unsaturated soil (Elrick and Reynolds, 1992).

The hole should be excavated using a screw-type or bucket auger. It should be cylindrical and have a flat bottom at least 20 cm above the water table (Reynolds, 1993). Specific factors that can affect the accuracy of the measurements with well permeameters include smearing, remoulding and compaction of the well surfaces during augering and gradual siltation of the well (Elrick and Reynolds, 1992; Reynolds, 1993). On clayey soils, scratching the bottom and side of the hole with a sharp-pointed instrument or metal brush normally alleviates the smearing and compaction caused during augering. On sandy soils, the collapse of the well during the measurement is the principal constraint to the use of this technique, whereas in silty soils the gradual siltation (clogging of the soil pores with silt particles) can be partially alleviated by protecting the bottom of the hole with coarse sand or fine gravel (Reynolds, 1993). Solar heating of the head space in the water reservoir can also affect the accuracy of the mariotte-based well permeameter, but this can be alleviated by performing the measurements under a tent or big umbrella. A further discussion of the method and procedures for calculating hydraulic parameters are given by Reynolds (1993), Elrick and Reynolds (1992) and Stephens (1996).

Unsaturated hydraulic conductivity

Most of the water movements above the water table in the field, including water and nutrient flux to plant roots, rainfall infiltration and leaching of nutrients through the soil profile, occur while the soil is unsaturated and are thus controlled by the unsaturated hydraulic conductivity. Unsaturated hydraulic conductivity can also be used to characterize changes in soil structure (Watson and Luxmoore, 1986; Wilson and Luxmoore, 1988; Ankeny *et al.*, 1990; White *et al.*, 1992), and this is an application of great relevance for agroforestry (see Fig. 11.4). Field measurements of unsaturated hydraulic conductivity are generally preferable to laboratory measurements if the site is sufficiently accessible, reasonably homogeneous, has a level topography, is not too stony and has predominantly vertical flow during drainage (Green *et al.*, 1986).

Tension infiltrometer or disc permeameter

This infiltrometer consists of a porous baseplate, which establishes hydraulic continuity with the soil through a nylon membrane. It is connected to a mariotte reservoir that supplies the soil surface with water at a constant and regulated tension. Tension infiltrometers normally operate in the range of 0 to –20 hPa. The data can be recorded manually or automatically (Ankeny, 1992; Elrick and Reynolds, 1992).

Tension infiltrometers have been used to characterize near-saturated hydraulic properties, sorptivity, macroscopic capillary length, characteristic pore size (Ankeny, 1992; Reynolds, 1993; Stephens, 1996), soil structural conditions at the soil surface as a function of short-term variations in weather conditions (White and Perroux, 1989), effects of tillage practices (Ankeny *et al.*, 1991; Messing and Jarvis, 1993) and root growth (White *et al.*, 1992). The near-saturated hydraulic conductivity and sorptivity obtained using tension infiltrometry have been used to distinguish infiltration through macropores from that through the soil matrix (Watson and Luxmoore, 1986; Wilson and Luxmoore, 1988; Messing and Jarvis, 1993). The method involves supplying water to the soil surface at different potentials to exclude a range of macropores from the flow, whose contribution to infiltration can thus be quantified.

A good soil surface preparation and hence good contact between the infiltration disc and the soil is essential. The supply membrane must be visible during the infiltration to permit examination for air leaks (Perroux and White, 1988). In older models the water tower was mounted on the infiltrometer disc, but these are now constructed separately, which makes the measurement more stable and accurate under windy conditions and avoids collapse of soil structure near saturation. For measurements near saturation the infiltration disc must be level; otherwise the potential varies

across the supply surface. Close to saturation, small changes in soil tension lead to dramatic changes of the infiltration rate and unacceptable errors in structured soils. The contact material can have a large influence on the pore water pressure head and hydraulic head gradient at the soil surface, and this can have a substantial impact on the validity of tension infiltrometer results (Reynolds and Zebchuk, 1996). A shortcoming of the technique is the time necessary to reach steady flow at low tensions in clayey soils, which can make manual recording impracticable. In this situation an automated infiltrometer should be used. As for Guelph permeameters, solar heating of the head space in the mariotte reservoir should be avoided by shading.

According to Reynolds (1993), the main factors affecting the accuracy of tension infiltrometer measurements are soil heterogeneity, soil collapse under the infiltrometer during the measurement, inadequate or changing hydraulic contact between infiltrometer and soil, and the hydraulic properties and thickness of the contact material.

Several methods have been reported for determining soil hydraulic parameters with infiltrometer data (Ankeny *et al.*, 1991; Elrick and Reynolds, 1992; White *et al.*, 1992; Logsdon and Jaynes, 1993; Simunek and van Genuchten, 1997). These methods differ in mathematical theory, type and number of infiltrometers used, and procedures of data collection. It is necessary to decide about the mathematical procedure for determining soil hydraulic parameters before starting the evaluations programme, because requirements concerning soil boundary parameters differ between methods. A modification of the double-ring method that allows the determination of unsaturated hydraulic parameters is discussed in the section on saturated hydraulic conductivity.

Indirect methods or pedotransfer functions

Field measurements of hydraulic properties are time consuming and labour intensive. Therefore, several attempts have been made to estimate these properties from readily available soil data such as particle size distribution, organic carbon and bulk density. An estimation method that describes soil water relationships based on other soil characteristics is called a pedo-transfer function (Tietje and Tapkenhinrichs, 1993).

Empirical approaches such as linear and non-linear optimization and neural network techniques have been used to solve specific problems in the determination of soil properties. However, this kind of approach requires some skills in mathematics and statistical analysis. Semi-empirical approaches have been used to estimate hydraulic properties from pore size (Mualem, 1976) and particle size distribution (Arya *et al.*, 1999).

The most common indirect approaches are the so-called parametric models, including those by Campbell (1974) and van Genuchten (1980).

Results to date have indicated that predictive models are relatively efficient for many coarse and medium-textured soils, but are unreliable for fine-textured and structured soils like structured clayey Oxisols (Tomasella and Hodnett, 1996). Eastham *et al.* (1988, 1994) and Chirwa *et al.* (1994a) have used these approaches in agroforestry research.

11.5 Estimating Topsoil and Subsoil Water Use with Stable Isotopes (*G. Schroth*)

The soil depth from which plants take up water is often of considerable interest, particularly where different species are growing together so that it determines the extent to which they compete with each other for the same water resources or complement each other by using water from different depths (see Section 11.1). Both hydrogen and oxygen possess stable, heavy isotopes (deuterium and ^{18}O, respectively), and in some situations the isotopic composition of the xylem water of a plant can be used to determine the depth of water uptake in the soil. The technique has recently been reviewed by Pate and Dawson (1999), and further methodological details are provided by Jackson *et al.* (1995).

A precondition for the application of this technique is that the isotopic composition of the soil water itself shows a change with depth that is sufficiently great to be detected reliably. Jackson *et al.* (1999) stressed the dynamic nature of the isotopic composition of the soil water and its depth distribution, which requires that site-specific, concurrent isotope profiles are established whenever xylem water values are analysed. This is partly due to the intra- and interannual variation of the isotopic composition of precipitation water, which showed a significant negative correlation between deuterium concentration and monthly rainfall at a Brazilian savanna site (Jackson *et al.*, 1999). In seasonally dry climates, the evaporation of water from the soil surface during the dry season causes the enrichment of water in the upper soil layers with the heavy isotopes, deuterium and ^{18}O, relative to water in deeper soil layers (Jackson *et al.*, 1995; D.M. Smith *et al.*, 1997b).

With the exception of mangroves, no isotopic fractionation occurs during water uptake and transport by plants. Consequently, the xylem water reflects the isotopic composition of the soil water at the depth of uptake, or the relative contribution of different water sources if water is taken up from more than one depth (e.g. groundwater and rain-derived water in the topsoil). However, isotopic fractionation does occur during transpiration, with preferential phase change from liquid to vapour for the lighter isotopes. As a consequence, the isotopic composition of the water in leaves and other transpiring tissues differs from that of the xylem and soil water. For woody plants, xylem tissue can be sampled from the

stem with an increment borer, or by mild vacuum extraction of twigs, branches and roots. Immature, unsuberized parts that are transpiring must be avoided (Jackson *et al.*, 1995; D.M. Smith *et al.*, 1997b; Pate and Dawson, 1999). For conifers, a method of extracting xylem water from logs with a displacement method has been described (Glavac *et al.*, 1990). For herbaceous species, non-chlorophyllous tissue at the interface between shoot and root has been used for water extraction (Dawson, 1993). D.M. Smith *et al.* (1997b) wrapped segments of the stem of millet (*Pennisetum glaucum*) plants in plastic film for at least 2 h before sampling them. In pot experiments, this method had been shown to avoid isotopic fractionation of xylem water by transpiration through the stem walls. For certain questions, the extraction and analysis of phloem sap can be of interest (Pate and Dawson, 1999). The isotopic composition of water samples can be determined with mass spectrometry, following either vacuum distillation (Jackson *et al.*, 1995) or direct equilibration (Scrimgeour, 1995). The ratio is expressed as δD or $\delta^{18}O$ (see Box 4.2 on p. 90 for delta notation), relative to standard mean ocean water (SMOW) or Vienna-SMOW.

Jackson *et al.* (1995) measured a gradient in the hydrogen isotope ratio of soil water with depth at the end of the dry season in the tropical forest of Barro Colorado Island, Panama. The gradient was most pronounced within the upper 30 cm of soil. Measuring the hydrogen ratio of the xylem water of different tree species, the authors concluded that evergreen species had access to water deeper in the soil than deciduous species, in agreement with the relatively high levels of physiological activity of the evergreen species during the dry season. Roupsard *et al.* (1999) studied the water relations of *Faidherbia albida* in a parkland agroforestry system in Burkina Faso. Comparing the oxygen isotope ratio of the xylem sap of the trees with that of the soil water at three times during the year, they concluded that at the beginning of the dry season, the trees took up water principally from the groundwater table at 7 m depth, whereas at the beginning of the rainy season, they probably took up water near the soil surface and may have competed here with the crops, although at a time when their transpiration was already strongly reduced by defoliation. At a third sampling occasion, at the end of the dry season, the depth of water uptake could not be clearly determined, as the isotope ratio in the soil water changed irregularly with depth and the ratio in the xylem sap could be explained with water uptake either from the subsoil (2–4 m) or from the groundwater table, or both.

Similar problems were encountered by Le Roux *et al.* (1995) in a study on the spatial partitioning of soil water between shrubs and grasses in a humid savanna in Côte d'Ivoire. They could explain observed oxygen isotope ratios in the xylem water of the shrub *Cussonia barteri* during the dry season with water uptake either from the upper 30 cm of soil or from

soil layers below 150 cm, which had a similar isotope ratio to the topsoil. A later study of plant water relations showed that much of the water uptake by the shrub during the dry season probably occurred from the subsoil, to which the grasses had no access (Le Roux and Bariac, 1998). This indicates that, in studies of soil water use, isotopic techniques should be complemented by other methods, especially the monitoring of soil and plant water status.

In a study using oxygen isotopes of water use by windbreaks in the Sahel, D.M. Smith *et al.* (1997b) demonstrated that the tree *Azadirachta indica* switched opportunistically during the year between the use of surface water and groundwater where the water table was not too deep, but took up water continuously from near the soil surface where the groundwater was not accessible, thereby increasing competition for water with crops. As an extension of the described methodology, Bishop and Dambrine (1995) used both the natural $\delta^{18}O$ gradient in the soil and a tritium (^{3}H) tracer, which was sprayed on the soil surface to localize soil water uptake by conifers with improved precision.

Hydraulic lift phenomena have also been studied with stable isotope techniques. Using the hydrogen isotope composition of soil and xylem water, Dawson (1993) showed that sugar maple trees (*Acer saccharum*) rooted through a hardpan in the subsoil and took up water from the groundwater layer, which was then released into the soil above the hardpan. As a consequence, the δD values of the water in the topsoil changed in a systematic way with increasing distance from the trees, with ratios close to those of the groundwater in the proximity of the trees and ratios close to those of rainwater at 5 m distance from the trees. Analysis of the xylem water of understorey plants, which had no access to the groundwater, revealed that these took up substantial quantities of the hydraulically lifted water from the soil when growing close to the maple trees, and this apparently improved their water relations and growth. This study and the employed methodology are of obvious relevance for agroforestry situations with deep-rooted trees and associated, shallower-rooted crops. For further applications of the methodology see Pate and Dawson (1999).

Chapter 12
Root Systems

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12.1 Synopsis

Root systems generally receive less research attention in agroforestry than they deserve, largely because of methodological difficulties associated with their study, especially the tedious and time-consuming nature of data collection. Despite these difficulties, a number of studies on tree root distribution in agroforestry systems are now available and have led to a reappraisal of the popularly held belief that trees are generally deep-rooted and so do not compete severely with crops for water and nutrients in the topsoil (Jonsson *et al.*, 1988). In fact, many trees do have deep roots, but they also have roots in the topsoil and acquire their nutrients and water where these are most available. In humid climates and during the wet months in seasonally dry climates, nutrients and water are usually most abundant and available in the topsoil, where tree roots often compete with crop roots for them (see Box 5.2 on p. 100). Such insights emphasize the need for a better understanding of the root ecology of tree–crop associations as a precondition for developing agroforestry practices that maximize beneficial interactions between trees, crops and soil, and minimize tree–crop competition.

The tension between expected soil-improving effects of tree roots on the one hand and competition with crop roots on the other is a specific feature of agroforestry associations (Schroth, 1995). Some competition is probably inevitable for efficient use of the available soil resources by the tree–crop association as a whole, but too much competition can easily cause economic failure of a system (see Section 5.1). The balance between

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beneficial and competitive effects of trees is especially critical on shallow, nutrient-poor and periodically dry soils, where soil resources are most likely to limit growth. On such sites, root processes in agroforestry associations require careful optimization, using the range of techniques that are briefly outlined below. In contrast, on sites without severe water and nutrient constraints, farmers may show little concern for root interactions between associated species. For example, on sites with sufficient rainfall and fertilization in Costa Rica, farmers use fast-growing and competitive tree species such as *Eucalyptus deglupta* as shade trees in coffee because of their favourable crown form, rapid shade establishment and low pruning requirements (Tavares *et al.*, 1999). On volcanic soils in East Java, farmers associate coffee with very fast-growing *Paraserianthes falcataria* trees (G. Schroth, personal observation). Root interactions between plants are also of less concern in fallow systems, where trees and crops are grown in sequence rather than simultaneously. Here, trees with large and potentially competitive root systems can be chosen for maximum soil improvement through soil organic matter enrichment, increase in soil porosity and deep nutrient capture without the danger of competition with crops growing in the same plot. However, in the small-scale patchwork of fallows and fields that is typical for some densely populated regions, root interactions may occur at the boundaries between adjacent crop and fallow plots and may affect a significant proportion of the cropped area (van Noordwijk, 1999).

On most sites, either water or nutrients limit growth for at least part of the year, and the possibility of competition between trees and associated crops for these resources requires consideration. Farmers can optimize root-related (and most other) processes in agroforestry associations through a combination of species selection, system design and subsequent management of the components (see also Section 5.1). Species selection in the present context refers to the genetic determination of the root characteristics of a species, whereas system design and management address in part the environmental factors that intervene in the expression of these characteristics. These are crucial, because the tree and other species in agroforestry systems will rarely be selected only because of their root characteristics (although these may influence the decision), but rather because of more immediate economic criteria such as fruit or wood production, ability to substitute mineral fertilizer through nitrogen fixation, or suitable crown architecture for the shading of light-sensitive crops. System design and management with respect to root processes can provide ways to increase the flexibility that a land user has to choose plant species, even if these do not have ideal root characteristics for a certain type of agroforestry practice (Schroth, 1996). Extending an earlier concept by Bowen (1984), the systematic use of species selection, system design and management for optimizing root functions has been termed root management (Schroth, 1995).

Tree species selection according to root characteristics may consider criteria such as the ability to rapidly develop a deep root system for nutrient recycling from the subsoil in fallows (Jama *et al.*, 1998), restricted lateral root development to reduce competition with crops (Laycock and Wood, 1963), a large root mass for physical improvement of compact subsoils (Schroth *et al.*, 1996), or relationships between root characteristics and nutrient availability in the soil after fallow (Schroth *et al.*, 1995b) (see also Fig. 5.2). Of particular interest for agroforestry are tree species that are not very competitive with crops but at the same time are soil-improving, a combination found, for example, with *Gliricidia sepium* (Schroth and Lehmann, 1995).

System design should be based on what is expected from the roots of a tree in a specific system. Fast-growing trees planted at narrow spacing in fallows can rapidly explore subsoil horizons (Jama *et al.*, 1998), but trees planted at wider spacing in tree–crop associations may be less efficient in this respect (see Section 8.1). If trees are needed for productive functions only, they may be planted in a different plot from the crops, but, if they are expected to improve the soil with their roots, these must have sufficient access to this soil. Planting trees on the plot boundary is a common strategy for reducing above- and below-ground interactions between trees and crops, although it may increase interactions with the neighbour's crops and create conflicts. An alternative method is to grow trees and crops in rotation as in improved fallows, but this limits the age that the trees may attain and, therefore, the range of products that they can produce. Fodder and firewood can often be produced in short fallows, but not timber, fruits and resins.

There is a wide range of management measures that influence root distribution and root functions in agroforestry systems. Soil tillage destroys the tree roots in the topsoil shortly before sowing the crops and gives these a temporary advantage, although tree roots grow rapidly back into the ploughed soil (Schroth *et al.*, 1995a). Tillage is a routine measure in systems with annual crops, with the exception of zero-tillage systems, so reducing root competition in this way does not cause extra costs. However, depending on the number and physical properties of tree roots in the topsoil, tillage can become more onerous and time-consuming in the presence of trees, especially when it is carried out with hand tools. Root pruning is a measure specifically designed to reduce root competition and may be carried out by deep ploughing along tree rows, possibly repeatedly during a cropping season, or by cutting off superficial tree roots with hand tools. The first of these options is restricted to mechanized agriculture and requires there to be sufficient space to pass between trees and crops with a plough, and the latter option is probably limited to a small number of trees. Competition for fertilizer may be reduced by restricting application to the zone of maximum root activity of the target crop or tree instead of

broadcasting it over the whole plot area. This may be combined with exclusion of competing plants such as weeds and cover crops by clean-weeding in the respective zone, as is often done with tree crops. Clean-weeding can, however, have non-target effects such as reduced mineralization of soil nitrogen in the weeded area close to the trees (Schroth *et al.*, 2000a, 2001c), so increasing the access of the tree to nutrients from fertilizer may lead to reduced access to soil-derived nitrogen. Additional fertilizer may reduce competition for the applied nutrients, but may also stimulate root growth, thereby increasing competition for other nutrients and water (Schroth, 1999). Shoot pruning of trees reduces their requirements for water and can be very effective in avoiding competition during drought periods (Smith *et al.*, 1998). However, impacts are species specific (Jones *et al.*, 1998) and pruning when trees are too young, or too severe and frequent pruning can induce shallow tree root systems (van Noordwijk *et al.*, 1991a) and may negatively affect tree survival and, in legume trees, nitrogen fixation (see Chapter 13). Herbaceous plant species with competitive root systems tend to reduce the lateral spread and increase the depth of the root systems of trees with which they are associated (Yocum, 1937; Neves *et al.*, 1998). It has recently been shown that simple grass strips can alter the root architecture of tree saplings by restricting their root growth in the direction of the grasses, a form of biological root pruning (Schaller *et al.*, 1999). Whether this translates into a more favourable root architecture of older trees requires further study. More detailed accounts of the effects of different management practices on tree root systems have been given in recent reviews (Schroth, 1995, 1999; van Noordwijk *et al.*, 1996).

Obviously, most root management measures have certain costs in terms of additional fertilizer input, labour or reduced tree growth, and these have to be outweighed by the benefits, such as improved crop growth and higher soil fertility, for them to be sensible options for farmers. The manipulation of root processes is not usually the only objective of a design or management decision, so roots are often 'co-managed' with other system properties. For example, the principal objective of tillage may be to suppress weeds but it may also reduce tree root competition; tree spacing and shoot pruning affect shade and other microclimatic factors as well as root distribution and density; and grass strips may control soil erosion and runoff and may produce fodder in addition to their potential effect on tree root distribution. In northern Nigeria, branch wood from trees was valuable because the demand for firewood was high, so tree pruning before crop planting controlled root competition while providing an economic return to the labour involved in pruning (Jones *et al.*, 1998).

Research needs

Agroforestry root studies have so far mainly concentrated on static analyses of tree root distribution, whereas dynamic root studies and process-oriented root research in agroforestry are still in their infancy. In the future, more efforts should go into the investigation of spatiotemporal patterns of root distribution and function, including the responses of root systems to management actions and the interactions between tree root systems and other soil biota such as fauna, microbes and other root systems. The following areas of root research are of particular interest.

- Spatial and temporal patterns of water and nutrient uptake from the soil as influenced by lateral and vertical root extension, root intensity including root length density, number of root tips and mycorrhization, and soil characteristics including soil physical properties, acidity, and water and nutrient content. Studies in this field may help in making practical management decisions, for example, through identification of zones and periods of competition between associated species, leading to efficient fertilizer placement, or through the identification of the potential for nutrient recycling from the subsoil, as well as by providing parameters for models of water and nutrient cycling.
- Effects of soil properties, either physical (compact horizons), chemical (acidity) or biological (pests, diseases, competing root systems), as well as management actions, such as tillage, fertilizer application and mulching, on root distribution.
- Effects of roots on the soil, especially through carbon inputs from root exudation and turnover, rhizosphere processes, creation of macropores and interactions with soil fauna.
- Methods of studying root distribution and functions in the field with little disturbance over extended time periods, including the *in situ* identification of the roots of associated plant species.

Further discussions of root processes and research methodologies in agroforestry are provided in Chapter 7 (root effects on nutrient leaching), Chapter 8 (lateral and vertical redistribution of nutrients), Chapter 10 (root effects on soil structure), Chapter 14 (mycorrhizas) and Chapter 15 (rhizosphere processes). The most recent and comprehensive text on root methods is that by Smit *et al.* (2000), which supersedes Böhm's (1979) classic work on the subject. Other useful sources of information on methods for measuring root systems and processes include Mackie-Dawson and Atkinson (1991) and several chapters in Lassoie and Hinckley (1991).

12.2 Methods for Studying Root Distribution

The analysis of root distribution in the field provides a basis for the study of root interactions with the soil and between associated plant species. Specifically, the processes of nutrient pumping from the subsoil or lateral nutrient capture by trees, which have been discussed in Chapter 8, require that the trees have access via their root systems to either deep or laterally distant soil which contains relevant amounts of nutrients and where these nutrients are sufficiently mobile to be taken up (van Noordwijk *et al.*, 1996). An investigation into these processes should thus comprise a comparative assessment of root, nutrient and water distribution in the soil, either in the vertical or in the horizontal direction from trees. The results of such an assessment may also serve as a basis for more detailed studies, for example by applying tracers into nutrient-rich subsoil horizons to which tree roots have access and measuring their uptake into the above-ground biomass (see Section 8.2). Also, at least some qualitative observations on the root distribution of trees and crops should be made in most agroforestry experiments as a precaution against interferences between neighbouring plots through far-reaching, lateral roots, which may invalidate the experimental results (see Section 3.2).

The principal methods for the analysis of root distribution in the field are the extraction of soil cores and the profile wall method. Excavation of representative sections of a plot can also be used, but this is much more labour intensive and is more appropriate when coarse roots need to be quantified, as in studies of total carbon accumulation in root systems (Akinnifesi *et al.*, 1999). Excavations are usefully combined with allometric techniques, such as relating (coarse) root mass to proximal root diameter (the diameter of main roots close to the trunk) or stem diameter (van Noordwijk *et al.*, 1996). There are also several indirect methods for studying the distribution of root activity, such as the analysis of water and nutrient distribution patterns in the soil (Box 8.1 and Section 11.2) or isotopic methods (see Sections 8.2 and 11.5). Estimating tree root distribution from architectural characteristics, such as by the application of fractal relationships or by estimating relative rooting depth of trees from the angles of main roots close to the stem, is still at an experimental stage (van Noordwijk *et al.*, 1996; Ong *et al.*, 1999).

Soil coring

Soil coring has the advantage of being less destructive than the preparation of profile walls, so that the same plot or tree can be used repeatedly for the same type of study, for example at the beginning and at the end of the growing season or during successive years. This allows the study of

dynamic root processes (see Section 12.4). After washing the roots from the soil cores, quantitative measurements of root mass or root length per unit soil volume can be made, and live and dead roots of different species can often be distinguished. Roots are usually washed from the soil over a sieve with 0.5 mm openings. Recent research with *Grevillea robusta* and maize roots indicated that this sieve size is adequate for studies of root mass, but that a 0.25 mm sieve may be required for accurate measurements of root length (Livesley *et al.*, 1999). To avoid bias due to contamination of roots by mineral soil, root mass is expressed as ash-free dry weight (Anderson and Ingram, 1993) or after conversion into a common carbon content of 45% (Schroth and Zech, 1995b).

Soil coring is most appropriate for root studies in superficial soil horizons. Coring equipment which allows the extraction of samples from depths greater than 1 m tends to be expensive and is not always available. In very stony or very hard soil, soil coring may be difficult or impossible, and the excavation of soil blocks of known volume can then be a suitable though more time-consuming substitute. For determining the volume of an irregularly shaped soil block, the hole can be filled with sand or lined with a plastic bag and filled with water.

Soil coring devices are usually relatively small in diameter (2–10 cm), so that only a limited volume of soil is sampled with each core (Caldwell and Virginia, 1989). As a consequence, the number of samples required to obtain representative root data may be large, especially in deeper subsoil horizons, where tree and crop roots usually occur at low densities (Mackie-Dawson and Atkinson, 1991). Soil coring for the assessment of subsoil root distribution in agroforestry may be most useful in dense and relatively uniform plant stands such as planted fallows. In systems with individual trees and pronounced spatial patterns, such as parklands or shade tree systems, careful design of the sampling scheme is necessary to produce meaningful results.

The identification of sampling points for soil coring should follow the same criteria as for soil fertility studies (see Section 3.4). As root distribution usually exhibits pronounced spatial patterns within agroforestry plots as a function of the position of the different plant species, random sampling from a whole plot is only appropriate in very dense and homogeneous stands. In more typical, heterogeneous agroforestry plots, either precise sampling positions are defined such as a number of fixed distances from a certain tree species, and replicate samples are collected from each position, or the plot is subdivided into more or less homogeneous strata from which random samples are taken (stratified random sampling, see Section 3.4). The first of these options gives more precise information about spatial patterns and can be used for geostatistical analysis or other interpolation methods, whereas the latter strategy gives more representative values for the plot as a whole without the need for spatial interpolation.

If the variability of the root data from individual samples is known, the number of replicate samples can be determined as described in Section 3.2. For objectives that require high precision in the measurement of root parameters, the necessary sample sizes will often be large, due to the large variability typically found in root studies. The effort for sample processing can be reduced drastically by bulking the replicate samples from the same position, homogenizing them (on a level surface, not in a bucket) and collecting a representative subsample. This can be done by dividing the sample into four quarters and retaining one quarter, which is again divided into four quarters and so on. The total sample and the subsample are weighed to determine the volume of the subsample from the known volume of the total sample. Tests with soil from a groundnut field showed that the root data from subsamples of 5–10% were sufficient to be representative of the total sample (Schroth and Kolbe, 1994).

Profile wall method

In comparison with root coring, the profile wall method has the advantage that no specialized equipment is required and that the roots are studied in their natural position. A trench is dug to a suitable depth, and the distribution of tree and other roots is studied on the trench wall, where roots intersecting the wall have been exposed. The roots can be counted manually, either *in situ* or in the laboratory after being traced on to transparent plastic film, or the profiles may be filmed with a video camera and the roots quantified by image analysis. This sometimes requires previous coloration of the roots to increase their contrast with the soil (Jorge, 1999). Profile walls give a good opportunity for studying the factors which influence root growth and distribution, such as soil cracks, earthworm channels or discontinuities in the sedimentation, or the effect of the roots on soil structure.

A significant drawback, however, with profile walls is that, even when applying great care in the preparation of the wall, many fine roots are lost, especially in clayey soils. For this reason, counting the root ends intersecting with the wall should not be seen as a quantitative method for the evaluation of root distribution without careful calibration against soil cores collected from different depths. In agroforestry associations, it could even produce misleading results because the finer roots of herbaceous species are much more likely to be lost during the preparation of the profiles than the coarser tree roots. There are also pronounced differences between tree species concerning the mechanical resistance of their fine roots and thus the likelihood of root loss. Furthermore, live and dead roots are difficult to distinguish in the profile wall, especially when the soil is dry. To avoid these difficulties, it is often advisable to quantify only coarse

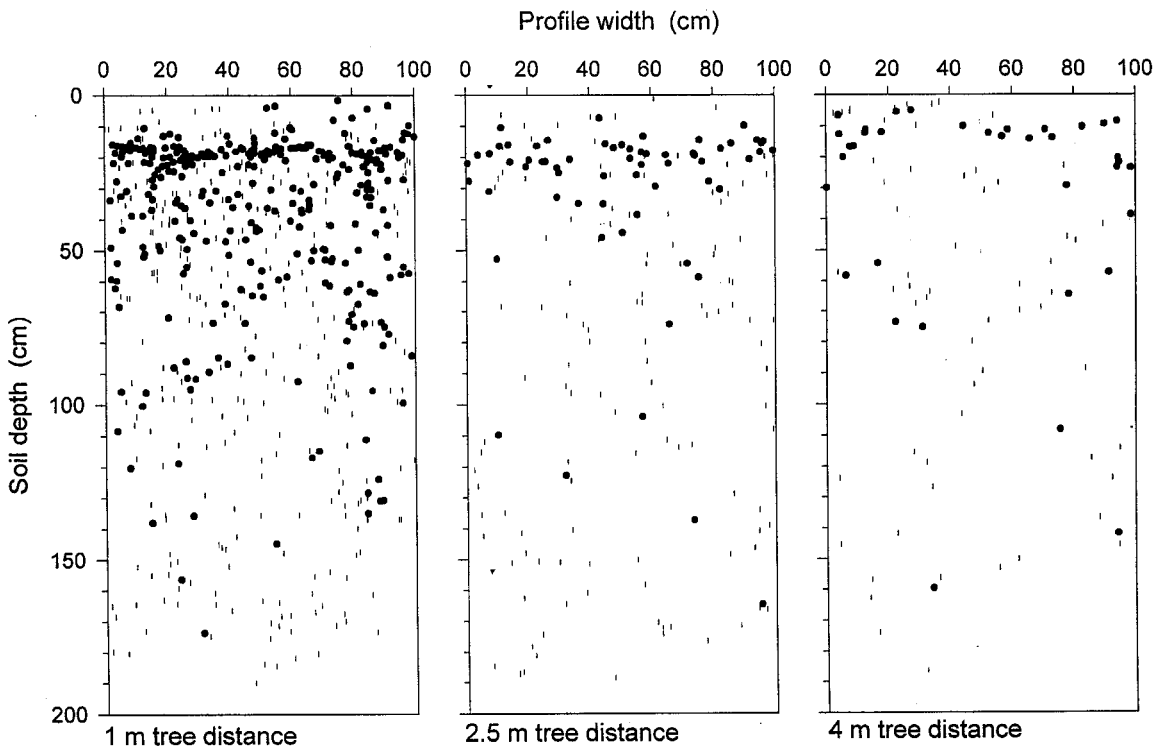


Fig. 12.1. Distribution of primary and secondary roots (dots and vertical lines, respectively) of oil palm (*Elaeis guineensis*) in a monoculture plantation in central Amazonia. The roots were marked on transparent sheets on profile walls at three tree distances. For roots < 2 mm see Table 12.1 (reproduced with permission from Schroth *et al.,* 2000a).

Table 12.1. Fine root length density of oil palm (*Elaeis guineensis*) at three tree distances in central Amazonia as measured with soil coring (roots < 2 mm, in cm cm^{-3}). For coarse roots see Fig. 12.1.[a]

Soil depth (cm)	Distance from the tree		
	1 m	2.5 m	4 m
0–10	12.8	2.8	1.3
10–20	5.1	0.8	1.7
20–30	5.8	1.1	1.4
50	3.1	1.0	0.6
100	0.6	0.2	0.2
200	0.7	0.2	0.1
300	0.1	–	–
400	0.0	–	–

[a] Reproduced with permission from Schroth *et al.* (2000a).

roots in the profile and to collect volumetric samples from different depths in the wall with a core or monolith sampler for the quantitative extraction of fine roots (Fig. 12.1, Table 12.1). Other disadvantages of the profile wall method are its destructive nature and the fact that root data measured on soil samples taken from a single profile wall in a plot are likely to be less representative for the plot than samples collected from several spots by means of soil coring.

Methods for horizontal root distribution

In studies of the lateral redistribution of nutrients within a system or landscape through tree roots, the horizontal root distribution especially in superficial horizons is of interest. In the case of tree rows such as boundary plantings, shelterbelts and hedgerows with close within-row spacing, the systematic pattern of tree root distribution is effectively reduced from three to two dimensions. Soil coring can then be an efficient strategy for studying root distribution. Soil cores are collected in lines parallel to the tree rows, and the cores from the same distance are mixed and subsampled for root extraction (Schroth *et al.*, 1995a). Before the sample collection, superficial trenches can be opened at different distances from the trees to obtain an idea of the tree root density to be expected and to assist in the definition of the exact sampling design (distances, depth increments, number of sampling points per distance and depth as influenced by root density and heterogeneity).

For individual trees, as in parklands and savannas, the tree root density may rapidly become too low and too patchy with increasing

distance from the trees to make soil coring an efficient strategy. Trenches can be dug either at increasing distances from a tree or as spiral trenches originating from the tree (Huguet, 1973), and the tree roots counted on the profile wall. Alternatively, or in addition, superficial coarse roots can be followed from the tree by successive excavation. Special attention should be given to patches of increased nutrient and water availability, such as ant and termite mounds, depressions receiving run-on water and eroded soil, and decomposing biomass (Mordelet *et al.*, 1996).

In studies of vertical and horizontal nutrient redistribution by tree roots, soil analyses for nutrients should accompany the root studies. The samples can either be collected separately or subsamples from the volumetric samples collected for the root studies can be used. The latter method is most appropriate if the roots are not extracted from the whole volumetric sample, but only from a subsample. Nutrient uptake from the subsoil is most likely when the topsoil is dry but the subsoil still moist. For this reason, monitoring the soil water content at different soil depths during wet and dry seasons is useful in studies of deep nutrient capture in seasonally dry climates.

12.3 Distinction of Roots of Different Species

In associations of several plant species, it is often of interest to know to which species certain roots belong. In many cases, the roots of a plant species are sufficiently distinct from those of all other species present at a site to be identified either in the profile wall or later in a Petri-dish under magnification. Typical criteria used are colour, diameter of the finest roots, surface properties, branching patterns and nodulation. In quantitative studies, care must be taken that all age classes of roots can be assigned to a species, as certain characteristics may not be equally visible in old and new roots. Sometimes it is not possible to distinguish the roots of every species, but it may still be possible to identify those of species groups, for example, to distinguish palms from dicotyledonous trees or legumes from non-legumes. Such distinctions may still be very useful in the interpretation of the data. The identification of species in the dead root fraction is often impossible. In the profile wall, some characteristics become less visible when the soil and the roots dry out, which can be avoided by shading and wetting the profile and in seasonally dry climates by carrying out such studies in the wet season.

In some cases, the biomass (including the roots) of associated species may differ in their contents of certain stable isotopes, so that the analysis of these isotopes in the root biomass of the individual species and in a mixed root sample can be used to determine the proportions of roots of different species in the sample. The proportions are obtained on a mass

basis and not on a root length basis. This is important, as species can vary considerably in the mass/length ratio of their roots (Caldwell and Virginia, 1989).

Plants following either the C_3 or the C_4 photosynthetic pathway differ in their $^{13}C/^{12}C$ ratio, which can therefore be used to determine the contribution of each group to a root sample collected from an association (Svejcar and Boutton, 1985) (see Box 4.2 on p. 90). Lehmann *et al.* (1998a) used this approach in an agroforestry system with *Acacia saligna* and sorghum (*Sorghum bicolor*).

In certain cases this method can be applied even if all plants at a site use the same photosynthetic pathway. In forests, the $\delta^{13}C$ values of the plant biomass gradually increase with plant size from understorey plants to canopy trees due to differences in stomatal opening and $^{13}C/^{12}C$ ratios of the carbon dioxide at different heights above the ground. Consequently, roots from tall trees can be expected to have higher $\delta^{13}C$ values than roots from smaller plants. This approach has been used to analyse the relative contribution of trees and understorey vegetation to the root mass in the soil down to 4 m depth in an Amazonian forest (Sternberg *et al.*, 1998), and it could probably be used in a similar way in agroforestry systems with a forest-like structure, such as forest gardens. Attention should be given to the observation of significant differences between the $\delta^{13}C$ values of roots and corresponding stems (Medina *et al.*, 1991).

Nitrogen-fixing species often differ from non-fixing species in their $^{15}N/^{14}N$ ratio (expressed as $\delta^{15}N$, see Chapter 13), so that this ratio could also be used to determine the contribution to mixed root samples of species in an association (Caldwell and Virginia, 1989). However, the differences are smaller than for the $^{13}C/^{12}C$ ratio and the ratios may overlap between nodulated and non-nodulated species (Guehl *et al.*, 1998). The ^{15}N contents of plants are influenced by factors such as mycorrhization, drought and plant nitrogen status (Handley *et al.*, 1999). Furthermore, there is considerable variability in space and time, the reasons for which include change in the ^{15}N content of soil with depth and landscape position as well as seasonal variations of nitrogen fixation (Handley *et al.*, 1994).

12.4 Methods for Root Dynamics, Production and Turnover

Root dynamics is a summary expression for temporal changes of root properties such as their mass, length and relative distribution in topsoil and subsoil. In comparison with static root data such as those obtained from a single measurement of root distribution, information on root dynamics is often of considerable additional value for the interpretation of agroforestry experiments. For example, in improved fallow systems, the ability of the trees to recycle leached nutrients from the subsoil is a function

of the progressive colonization of the subsoil by tree roots (Jama *et al.*, 1998), and the speed of tree root growth in the subsoil may be one of the factors that determine the minimum time under fallow needed for effective improvement of topsoil fertility. In simultaneous agroforestry systems, the root dynamics of associated species may influence their below-ground interactions: if phases of maximum root growth occur at the same time for different species, below-ground competition is likely to be high, whereas a certain lag in these phases for associated plants may indicate a degree of complementarity in the use of soil resources (Campbell *et al.*, 1994; Muñoz and Beer, 2001). The analysis of temporal patterns of plant root growth can also show whether phases of increased root growth coincide or are out of synchrony with phases of increased nutrient availability in the soil, such as from nitrogen mineralization or fertilizer application, and this may influence the efficiency with which nutrients are taken up (see Section 6.1). Root dynamics may also reflect the response of plants to management actions, such as shoot pruning (Nygren and Ramírez, 1995; Schroth and Zech, 1995a) or fertilizer application (Campbell *et al.*, 1994), which may influence the competitive balance among associated species.

Root turnover is a specific aspect of root dynamics referring to the fraction of a root system that is renovated during a certain time period (commonly a year) through the death of some roots and their replacement by new root growth. There is no universally accepted definition of root turnover. It has been defined by different authors as either the cumulative root production or the cumulative root mortality during a study period (which is numerically the same as long as live root mass is similar at the beginning and the end of a study period), divided by either the average, maximum or minimum root mass during this period (Gill and Jackson, 2000). Defining root turnover as the cumulative root production divided by the maximum root mass has the advantage that it yields a turnover of 1 for an annual crop that maintains all its roots until the end of the growing season (Gill and Jackson, 2000). However, using maximum (or minimum) instead of average root mass as a basis for the calculation has two disadvantages: (i) the generally greater uncertainty in the measurement of extreme than of average values, and (ii) the risk of missing the true maximum when sampling the roots. Therefore, calculating root turnover as cumulative production divided by average live root mass is likely to yield results that are less prone to experimental error. Average root mass should be calculated by suitable weighting according to what sampling intervals have been used. In any case, root turnover values should always be reported together with the calculation method used and should be accompanied by data on root production and mortality as well as average, maximum and minimum root mass during the study period to facilitate comparisons between studies.

Root turnover may occur naturally or may be a response to

management interventions such as soil tillage or shoot pruning (see Vogt and Bloomfield, 1991, and Eissenstat *et al.*, 2000, for discussions of the mechanisms involved in natural root senescence and turnover). Root production and turnover are of interest in agroforestry studies because the investments of carbon and nutrients into plant root systems affect the resources that are available for above-ground growth and production. Furthermore, root turnover is a mechanism through which plants replenish the soil with organic matter, supply carbon and energy to the soil biomass and release nutrients into the soil.

Several methods are available for studying root dynamics in agroforestry systems. Some of these may be used to obtain quantitative or semiquantitative information on root production and turnover, whereas others are useful for qualitative purposes. Quantitative methods tend to be very demanding in time. Also, root studies are generally characterized by relatively large variability, which is partly caused by the spatiotemporal heterogeneity of the soil environment and its usually unknown effect on the root systems of different plants. Careful definition of research objectives and corresponding selection of methods in accord with the available resources, especially labour, are particularly important in research on root dynamics. A recent review of research methods can be found in Vogt *et al.* (1998).

Sequential soil coring

This is a frequently used technique in research on root dynamics. It consists of the repeated collection of volumetric soil samples in the field, usually with a cylindrical soil corer, from which the roots are extracted by washing over a sieve, then separated into live and dead roots, diameter classes and plant species as required, and quantified in terms of their ash-free dry weight or length (see Sections 12.2 and 12.3). As mentioned before, the method is only suitable for relatively fine roots (smaller than about 5–10 mm), but these are also the most dynamic root fraction. Sequential coring can provide a quantitative picture of fine root systems at different sampling times, which may describe seasonal or phenological patterns and be related to water and nutrient uptake (Kummerow *et al.*, 1982; Singh and Srivastava, 1985; Schroth and Zech, 1995a; Lehmann *et al.*, 1998a; Jose *et al.*, 2000). Sequential soil coring data have been used for calculating root production and turnover in agroforestry by Cuenca *et al.* (1983), Schroth and Zech (1995b) and Lehmann and Zech (1998). There are also several root production studies from tropical forests using sequential soil coring (Kummerow *et al.*, 1990) or sequential excavation of soil blocks (Singh and Singh, 1981; Srivastava *et al.*, 1986; Khiewtam and Ramakrishnan, 1993). Dynamics of mycorrhizal fungi have also been studied with this method (Fogel and Hunt, 1983).

The collection of soil cores should be timed so as to include expected minima and maxima as well as critical situations in the root development of the system and its components. For example, in a system with trees and annual crops, samples may be collected before sowing the crop (to characterize the tree-only situation), during early crop development (when the soil may be dominated by tree roots), during maximum root development of the crop (when the crop roots may be more abundant than the tree roots), and after crop harvest when the crop roots have died and are decomposing in the soil. In a system where trees are pruned as part of their management, appropriate sampling times may be before the first pruning at the onset of the rainy season (presumably maximum tree root development) and then at monthly or bi-monthly intervals to check for root decline caused by the pruning treatment. In systems with perennial crops, such as coffee or cocoa with shade trees, the most pronounced contrasts would be expected between samples collected during the wet and the dry season (Cuenca *et al.*, 1983), or between samples collected during vigorous vegetative growth and when the crop is weakened by reproduction at the end of the harvest season.

To increase the probability that temporal changes of root properties can be seen in the data, the influence of spatial heterogeneity should be reduced by carefully standardizing the sampling protocol. For example, samples can always be taken from the same distances or distance ranges from a tree row or a number of shade trees, using the same trees for each sampling. The sampling depth is best defined on the basis of preliminary investigations of vertical root distribution in the field, taking into consideration that the inclusion of subsoil samples may greatly increase the effort required for sample collection in the field, although the time required for sample processing is usually much less for subsoil than for topsoil samples. Pooling several soil cores from similar sampling positions and depths and subsampling for root extraction is an efficient way for reducing the processing time (Schroth and Kolbe, 1994; Section 12.2) and may often be the only way to cope with the large number of samples collected during studies of root dynamics.

Estimating fine root production and turnover

Several methods have been developed for estimating fine root production and turnover from sequential soil coring data. These calculations are based on the following equations (Publicover and Vogt, 1993; Vogt *et al.*, 1998):

$$\mathrm{LFR}_{t2} = \mathrm{LFR}_{t1} + P - M \tag{12.1}$$

$$\mathrm{DFR}_{t2} = \mathrm{DFR}_{t1} + M - D \tag{12.2}$$

where LFR and DFR are the biomass of live and dead fine roots, respectively, t is time period, and P, M and D are production, mortality and disappearance, respectively, between times t_1 and t_2. These equations can be combined to yield the basic equation of net primary production of fine roots (P):

$$P = \mathrm{LFR}_{t2-t1} + \mathrm{DFR}_{t2-t1} + D_{t2-t1} \quad (12.3)$$

To calculate total below-ground carbon allocation, terms for carbon losses such as exudation, respiration and allocation to mycorrhizal fungi would have to be added to root production (Vogt *et al.*, 1998).

Calculations of root production make use of either one, two or all three of the terms in Eq. 12.3. The simplest method (max–min method; Publicover and Vogt, 1993) consists of the summation of increases in LFR between sampling dates. If the data show a single maximum and minimum, as, for example, with an annual crop or perennial vegetation in a strongly seasonal climate, then production is calculated by subtracting the minimum from the maximum LFR. Implicitly, this method assumes that root growth and death are separated in time and, if both processes occur simultaneously, production tends to be underestimated (but see discussion of other sources of error below).

A second calculation technique, called the balancing transfers method (Fairley and Alexander, 1985), considers changes in both LFR and DFR according to a decision matrix (Table 12.2). However, it does not consider the decomposition of dead roots during a sampling interval and may, therefore, still underestimate total root production. In fact, for a hypothetical data set where LFR and DFR are constant through time, both

Table 12.2. Decision matrix for estimating root production (P), mortality (M) and disappearance (D) from soil coring data of live and dead fine roots (LFR and DFR, respectively) according to the balancing transfers method. Annual estimates are made by summing the estimates from all sampling intervals within a year.[a]

		Live roots decrease	
	Live roots increase	ΔDFR > ΔLFR	ΔLFR > ΔDFR
Dead roots increase	$P = \Delta\mathrm{LFR} + \Delta\mathrm{DFR}$ $M = \Delta\mathrm{DFR}$ $D = 0$	$P = \Delta\mathrm{LFR} + \Delta\mathrm{DFR}$ $M = \Delta\mathrm{DFR}$ $D = 0$	$P = 0$ $M = -\Delta\mathrm{LFR}$ $D = -\Delta\mathrm{LFR} - \Delta\mathrm{DFR}$
Dead roots decrease	$P = \Delta\mathrm{LFR}$ $M = 0$ $D = -\Delta\mathrm{DFR}$	$P = 0$ $M = -\Delta\mathrm{LFR}$ $D = -\Delta\mathrm{LFR} - \Delta\mathrm{DFR}$	

[a]Reproduced with permission from Fairley and Alexander (1985).

the max–min and the balancing transfers method would indicate zero production (Kurz and Kimmins, 1987), although this situation could in theory be the result of a perfect equilibrium between root production, mortality and decomposition. Root production could also be underestimated if sampling does not coincide with seasonal peaks and troughs in root mass (Publicover and Vogt, 1993). In comparisons of agroforestry with annual cropping systems, this bias is particularly problematic because root growth and mortality are likely to be more separated in time for annual plants than for trees, and root turnover could thus be more strongly underestimated in the agroforestry plots than in the agricultural control plots (Schroth and Zech, 1995b).

The third and, theoretically, the most satisfactory approach to estimate root production is the compartment flow method (Santantonio and Grace, 1987; Publicover and Vogt, 1993), which considers all three terms of Eq. 12.3. With this method, mortality (M) is calculated from changes in DFR and a decomposition constant k, assuming an exponential decomposition of the dead root mass. Production and decomposition are then calculated by transforming Eqs 12.1 and 12.2 above (Publicover and Vogt, 1993):

$$M = kt[\mathrm{DFR}_{t2} - \mathrm{DFR}_{t1}e^{(-kt)}] / [1 - e^{(-kt)}] \quad (12.4)$$

$$P = \mathrm{LFR}_{t2} - \mathrm{LFR}_{t1} + M \quad (12.5)$$

$$D = \mathrm{DFR}_{t1} - \mathrm{DFR}_{t2} + M \quad (12.6)$$

The decomposition constant k is related to the disappearance rate DR (i.e. the fraction of dead roots that disappears during a certain time interval such as a month) by:

$$k = -\ln(1 - \mathrm{DR}) \quad (12.7)$$

Equation 12.4 can be modified if root decomposition is known to differ from the simple exponential model. In theory, the compartment flow method allows accurate estimation of root production even when root growth, mortality and decomposition occur simultaneously. The principal problem with this approach is the need for an accurate decomposition rate for fine roots, which is difficult to obtain (see Section 6.3). Under agroforestry conditions, the decomposition rate would depend on the relative contribution of tree and crop roots, including its seasonal fluctuations, to the dead root pool, as these roots are likely to differ in their decomposition dynamics. Unfortunately, the method is relatively sensitive to errors in the decomposition rate. It may, therefore, be useful to provide production estimates for a range of decomposition rates (Schroth and Zech, 1995b). The method is also sensitive to misidentification of live and dead roots, which are often not easy to distinguish, especially for tree species with dark roots, and to loss of dead roots when processing the

samples. However, modelling results indicate that the production estimates remain more accurate than those of the other two calculation methods unless the processing error becomes quite large (Publicover and Vogt, 1993). Whenever possible, the compartment flow method should be used for calculating root production from sequential soil core data, possibly in combination with the balancing transfers method for comparison.

As indicated above, the max–min and the balancing transfers methods have an inherent tendency to underestimate root production because they ignore the simultaneous occurrence of root production, mortality and decomposition. However, there is another source of error with these methods which may lead to an overestimation of root production. This error arises from the summation of random increases in root mass between sampling periods. It increases with increasing sampling frequency and is especially severe for sites where there are no pronounced seasonal changes in live and dead root mass. To avoid this error, it is recommended to use only statistically significant changes in root mass for production calculations with these two methods (Vogt *et al.*, 1998). This is a serious limitation because it requires the separate processing of many replicate root samples which could otherwise be pooled and subsampled to increase the precision of the measurement that can be achieved per unit processing time (Schroth and Kolbe, 1994). In some cases this problem can be circumvented because it is known, or at least it is very likely, that roots have turned over, even if differences between sampling dates are not significant at a given level of replication. This would be the case when tree roots show pronounced seasonal fluctuations in accord with weather or management actions such as pruning, in systems with annual crops whose roots necessarily die after the harvest (Schroth and Zech, 1995b), or when additional observations (e.g. with rhizotrons, see below) indicate root turnover between sampling dates (Vogt *et al.*, 1998). However, where such evidence is not available and root data show no pronounced seasonal patterns, as for perennial crops or trees in climates without pronounced seasons, the max–min and the balancing transfers methods should not be used for production calculations. Modelling results indicate that the compartment flow method is not prone to this source of error (Publicover and Vogt, 1993), and it is therefore not necessary to restrict root production calculations to significant changes in live and dead root mass. The compartment flow method is ideally combined with the pooling–subsampling method to reduce processing time (Schroth and Kolbe, 1994). As indicated before, however, further research on root decomposition rates for different trees and crops as influenced by soil and climatic conditions and their mathematical description is required to make full use of the potential of this method.

Sequential excavation

As soil coring is only suitable for sampling fine roots, other methods are required when coarse roots are also of interest, such as in studies of total carbon accumulation or progressive soil exploration by coarse lateral or vertical roots. Dynamics of coarse roots can be studied by sequential excavation of trees of different ages, which may be combined with soil coring for quantification of fine roots. Sequential excavation was used, for example, by Yocum (1937) for coarse and fine roots in his studies of apple tree root development as influenced by associated crops and soil management practices. After suitable calibration, allometric techniques or simply the root–shoot ratio can be used for estimating coarse root biomass of trees (van Noordwijk *et al.*, 1996). These techniques have rarely been used in agroforestry because of the considerable work involved and the focus on fine roots in most studies. They could, however, become more relevant with increasing attention given to the potential of trees to increase carbon sequestration in agricultural systems.

Root ingrowth method

This method is best suited for comparative studies of root growth for different time periods, sites or experimental treatments, although the method has also been used for estimating root production in tropical forests (Jordan and Escalante, 1980; Kangas, 1992) and shaded tree crop plantations (Muñoz and Beer, 2001). A certain soil volume (usually a core, but sometimes a soil block) is removed and replaced by root-free soil in a mesh bag, so that root ingrowth during the incubation period into a defined soil volume can be determined. The mesh bag can be made of nylon or metal netting with an approximately 5 mm mesh (Steen, 1991; Muñoz and Beer, 2001). The soil in the ingrowth core is either root-free soil from the same site, or a standard substrate such as sand or vermiculite. If soil from the site is used, live and dead roots are removed as far as possible by dry sieving. The use of a polyethylene sheet, charged with static electricity by rubbing, can help in the removal of fine root fragments (Muñoz and Beer, 2001). These procedures will not produce a completely root-free soil, so root residues should be extracted from a subsample for correction of the final data of root ingrowth (Persson, 1990). Incubation periods of 1–12 months have been used in humid tropical (Cuevas and Medina, 1988; Raich *et al.*, 1994; Muñoz and Beer, 2001) and Mediterranean climates (Fabiao *et al.*, 1985). Depending on the research objective, either all cores are collected after a fixed incubation period, or sets of cores are collected after increasingly long incubation periods (e.g. 3, 6, 9 and 12 months) to study the progress of root ingrowth into the

cores. In the absence of site- and species-specific experience with the method, the latter approach is probably preferable. With the ingrowth technique, Fabião *et al.* (1985) studied the seasonal dynamics of root growth in *Eucalyptus globulus* plantations in Portugal, and Muñoz and Beer (2001) studied root growth dynamics and turnover in shaded cocoa plantations in Costa Rica.

The ingrowth method is well suited for studying the effect of experimental soil manipulations on root growth. For example, Cuevas and Medina (1988) and Raich *et al.* (1994) demonstrated limitation by certain nutrients for forests in Amazonia and Hawaii, respectively, through increased root ingrowth into cores with standard growth media to which these nutrients had been added. With a similar design, McGrath *et al.* (2001) could not clearly detect phosphorus deficiency in an Amazonian agroforestry system despite very low soil phosphorus availability. Steen and Hakansson (1987) demonstrated reduced root growth of annual crops into cores with increasing soil compaction.

A disadvantage of the method is that, unlike sequential soil coring, it does not provide information on root distribution in the soil. If this information is required, the method has to be combined with another method, such as soil coring (Muñoz and Beer, 2001) or the profile wall method.

If quantitative growth data are needed, a potential problem is the difficulty of creating conditions in the core which are similar to those in the surrounding soil with respect to factors such as soil structure (and thus water content), nutrient mineralization and the presence of competing roots. This is much easier to achieve in a recently tilled topsoil of an agricultural field than in a plot under permanent vegetation. In agroforestry studies with several contrasting plant species, such as annual crops and trees, artificial growth conditions in the cores could affect plant species differently, thereby introducing bias.

If ingrowth cores are used for measuring root production, information on live roots, dead roots and root decomposition should be taken into consideration, as outlined above for sequential soil coring (Steen, 1991). Several authors found reasonable agreement of production values obtained with the ingrowth and sequential soil coring methods (Persson, 1983, for a boreal pine forest; Hansson and Andrén, 1986, for a perennial grass ley; Symbula and Day, 1988, for a swamp forest), whereas others found substantial differences in fine root production (Neill, 1992, for a prairie marsh; Makkonen and Helmisaari, 1999, for a boreal pine forest). Until research in tropical agroforestry systems provides evidence that (or under what conditions) root production estimates from ingrowth cores are reliable, it is recommended that this technique is used for comparative purposes or in combination with other methods for measuring root production.

Minirhizotrons

Minirhizotrons are a non-destructive method for the *in situ* observation and quantification of roots in the soil. They consist of a transparent access tube, such as a glass or acrylic tube, or a hole in the soil, through which images of roots growing at the interface with the soil can be obtained with a fibre optic probe, miniaturized video camera, or simply a mirror and a camera with a macro-lens (Mackie-Dawson and Atkinson, 1991; van Noordwijk *et al.*, 1996). Johnson *et al.* (2001) provide a recent review of the technique.

Minirhizotrons have been used for collecting data on root length distribution, root dynamics (growth, senescence and decomposition), root properties (branching patterns and suberization) and interactions of roots with other organisms (herbivory, parasitism and symbioses such as root nodules formed with rhizobia). Due to their non-destructive nature, they allow roots to be monitored through different seasons, wetting–drying cycles, phenological cycles of a plant, or in response to management interventions such as shoot pruning or fertilization (Ball-Coelho *et al.*, 1992; Kätterer *et al.*, 1995; van Noordwijk *et al.*, 1996; Price and Hendrick, 1998; Vogt *et al.*, 1998). If roots of different species can be distinguished, the technique allows comparative studies of root dynamics of associated trees and crops or pastures in agroforestry associations, which may give important information on below-ground interactions between species (Campbell *et al.*, 1994). Often, however, it is not possible to distinguish roots of associated species (Hendrick and Pregitzer, 1996). Several software packages are available for analysing root data from minirhizotron observations (Vogt *et al.*, 1998).

When working with minirhizotrons, an important consideration is how to avoid artefacts due to altered growth conditions of the roots at the interface between minirhizotron and soil. In sandy, uniform, well-packed soil, the presence of rigid observation tubes does not seem to influence root growth (Vos and Groenwold, 1987; Ephrath *et al.*, 1999), although preferential root growth at the soil–tube interface may occur in finer-textured soils. In compact soil, this may lead to deeper root growth along the tubes than in the bulk soil (Vos and Groenwold, 1987). To reduce the problem of gap formation at the interface between rigid observation tubes and soil, several types of inflatable minirhizotron tubes have been tested. Gijsman *et al.* (1991) described a system consisting of a rubber tube, actually a modified motorcycle inner tube, which exerts a small pressure on the surrounding soil when inflated, thereby avoiding gaps at the interface, and is removed when observations are made. Advantages include the better visibility of the roots, which are not obscured by an imperfectly transparent tube, and the elasticity of the tube, which is important in swelling and shrinking soils (Gijsman *et al.*, 1991; van Noordwijk *et al.*, 1996).

Another factor to be considered is the installation angle of the tubes. Root growth along the side of minirhizotrons has been observed with vertically installed, rigid tubes. Sometimes, but not always, this can be avoided by installing the tubes at an angle of 45° to the soil surface (Mackie-Dawson and Atkinson, 1991). However, in studies of deep root systems, as with many trees, installation angles other than vertical are impractical (Ephrath *et al.*, 1999). In a study with *Acacia saligna* and wheat in a sandy, disturbed soil, Ephrath *et al.* (1999) found no difference in root length density measured with tubes placed vertically or at an angle of 45° in the soil. As discussed above, larger effects of the installation angle would be expected in more clayey soils. In studies with shallow-rooted crops, horizontal installation of minirhizotrons may be advantageous because it leads to more images per soil depth, thereby reducing variability of mean values (Dubach and Russelle, 1995) and allowing the analysis of spatial patterns. Johnson *et al.* (2001) provide a detailed discussion of installation methods for rhizotron tubes and recommend a waiting period of 6–12 months between installation and data collection in order to avoid artefacts caused by soil disturbance.

Light can negatively affect root growth, even at low intensities, and so its entrance into the tubes needs to be avoided (Caldwell and Virginia, 1989). Also, temperature effects should be avoided by insulating exposed ends of rhizotron tubes above the soil surface (Samson and Sinclair, 1994).

The minirhizotron technique is well suited for comparative measurements of root dynamics in different seasons or experimental treatments. Repeated observations of the same root can provide information on root longevity and turnover. Johnson *et al.* (2001) recommended sampling intervals of 2 weeks or less in such studies in order to reduce underestimates of root turnover, which occur when roots appear and disappear between two sampling events. Difficulties have been encountered when attempting to transform minirhizotron readings into quantitative values of root length or root mass per unit bulk soil. Calibrations of minirhizotron observations against soil coring data produced relationships that varied with plant species and phase of crop development (Ball-Coelho *et al.*, 1992; Merrill and Upchurch, 1994; Samson and Sinclair, 1994). Furthermore, for both herbaceous crops and trees, correlations between these methods tend to be poorest near the soil surface, where root length is often underestimated by the rhizotron technique (Ball-Coelho *et al.*, 1992; Samson and Sinclair, 1994; Ephrath *et al.*, 1999). This is problematic, because most roots are usually concentrated here. A theoretical calibration approach for estimating root length from minirhizotron data also produced contradictory results (Merrill and Upchurch, 1994). More encouraging results were recently obtained for estimating root mass of maize and two temperate tree species from minirhizotron observations of root surface area (Jose *et al.*, 2001).

Despite these difficulties, the minirhizotron technique can be useful in the estimation of root production if the turnover values obtained from repeated observations of roots at different depths are combined with absolute root mass data as obtained, for example, with soil coring at one or several points in time. Studies that have used this approach have been reviewed by Hendrick and Pregitzer (1996).

Nutrient fluxes associated with root turnover

Nutrient fluxes through fine root systems of trees are often calculated by multiplying estimates of root production with nutrient concentrations in root samples, assuming that there is no retranslocation of nutrients from senescing roots (Burke and Raynal, 1994; Lehmann and Zech, 1998; Muñoz and Beer, 2001). Available evidence on this topic is contradictory. A study by Nambiar (1987) suggested little translocation of nitrogen, phosphorus, potassium and magnesium from senescing fine roots of *Pinus radiata*, but other studies have indicated significant retranslocation of nitrogen and phosphorus from senescing roots of northern conifers (Meier *et al.*, 1985; Ferrier and Alexander, 1991). A recent analysis of a large data set of nutrient concentrations in live and dead roots found no evidence for retranslocation of nitrogen, but indicated an average retranslocation of about 30% of phosphorus and potassium from senescing fine roots. For magnesium the average tissue concentration decreased by 25% between live and dead fine roots, but the difference was not significant (Gordon and Jackson, 2000). These results suggest that the turnover of phosphorus, potassium and perhaps magnesium in fine roots may be overestimated if calculated as indicated above, although estimates of nitrogen turnover in fine roots may in many cases be reliable. Further research is needed to confirm this. If roots of annual crops die after harvest or tree roots are sloughed off by tillage, retranslocation of nutrients obviously does not occur.

Chapter 13
Biological Nitrogen Fixation

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13.1 Synopsis

Trees can increase nutrient inputs and reduce nutrient losses through a variety of processes. Biological nitrogen fixation is prominent among these as it is the only truly renewable source of nutrients in agroforestry or agriculture as a whole. Many of the trees used in agroforestry are legumes as the ability to fix nitrogen allows them to grow rapidly in nitrogen-depleted soils. Thus one of the principal roles of nitrogen-fixing trees in agroforestry is to improve soil fertility, athough many legume trees have multiple uses for fodder, fuelwood, fruits and timber. Legume trees are thus found in almost all types of agroforestry systems: as shade trees in perennial crops, in improved fallows, in hedgerow intercropping systems, as erosion barriers, live fences, isolated trees in parklands and fodder trees.

Not all legumes can nodulate and fix nitrogen, and the taxonomy of the *Leguminosae* is a good guide to which legume trees are nitrogen fixers. Nitrogen-fixing symbioses are also formed between non-legume trees and actinomycetes (*Frankia* spp.), which are termed actinorhizal symbioses (Giller, 2001). The most important of these are the *Casuarina* spp. from Australasia, which are used for soil stabilization, as windbreaks and for poles and fuelwood throughout the tropics. The actinorhizal symbioses have been less studied in agroforestry than leguminous trees, but the methods outlined here can equally well be applied to the study of either type of symbiosis. A full list of nitrogen-fixing trees also includes larger species of cycads, which form symbioses in their leaf axils with blue-green algae (*Cyanobacteria*), but these are of no importance in agroforestry.

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Nodulation and nitrogen fixation in the *Leguminosae*

The *Leguminosae* are a large and diverse family of flowering plants composed of some 18,000 species, ranging from huge, long-lived forest trees to tiny, annual, herbaceous forbs. All legumes have nitrogen-rich tissues, irrespective of whether they can fix nitrogen, and it is now thought that the nitrogen fixation ability evolved to satisfy the demand for nitrogen, rather than nitrogen fixation resulting in the high tissue concentrations of nitrogen, which had often been assumed earlier (see McKey, 1994; Giller, 1997). Those legumes, which are not able to fix nitrogen, also have a large tissue nitrogen demand, which they satisfy either by competing actively for soil nitrogen or by growing more slowly and conserving their nitrogen within the plant. A further major consequence of the large tissue concentrations of nitrogen in the *Leguminosae* is the wide variety of secondary metabolites which are produced to protect legume tissues from predation (Waterman, 1994). Among these secondary metabolites the polyphenols, and particularly the condensed tannins, play a major role in regulating nitrogen release from legume tissues in decomposition (see Sections 6.1 and 6.4) and during ruminant digestion (Giller, 1997).

The *Leguminosae* are divided into three subfamilies, the *Caesalpinioideae*, the *Mimosoideae* and the *Papilionoideae*, and these subfamilies provide a useful guide as to whether the legumes are able to nodulate and fix nitrogen. The *Caesalpinioideae*, of which only 23% form nodules (de Faria *et al.*, 1989; Sprent, 2001), are considered to be the most primitive group. Within the *Caesalpinioideae*, nodulation is restricted to a few tribes, the most notable of which is *Cassieae,* which appear to form a bridging group between the non-nodulating and nodulating legumes. Within this tribe all members of genera *Cassia* (*sensu stricto*) and *Senna* are unable to nodulate. The genus *Senna* includes several important agroforestry species, such as *S. siamea* and *S. spectabilis*, which have been widely tested in hedgerow intercropping and were earlier assigned to the genus *Cassia* (Irwin and Barneby, 1981). Closely related to the genera *Cassia* and *Senna* is the genus *Chamaecrista*, in which all the species examined nodulate and fix nitrogen. The range of nodule morphology within the genus *Chamaecrista* is striking, with some species having rather primitive nodule structures, in which the bacteria are maintained in persistent infection threads within the nodule cells, whereas in other species fully differentiated bacteroids are released within peribacteroid membranes in the host cell cytoplasm (Naisbitt *et al.*, 1992). Persistent infection threads are also found in the only non-legume tree genus, *Parasponia* (*Ulmaceae*), which can form nodules and fix nitrogen with rhizobia (Trinick and Hadobas, 1988; Becking, 1992).

Almost all legumes in the other two subfamilies, the *Mimosoideae* (90%) and the *Papilionoideae* (97%), can form nodules with rhizobia and fix nitrogen (de Faria *et al.*, 1989). The exceptions in the *Mimosoideae* appear

to be cases where loss of nodulation and nitrogen-fixation ability has occurred in species which are found in very arid environments (e.g. some *Acacia* spp.), presumably due to the sensitivity of nitrogen fixation to drought (Odee and Sprent, 1992; Sprent, 1994). Members of the *Papilionoideae* which do not nodulate are largely those found in primitive tribes.

Useful reference texts with information on nodulation and nitrogen fixation in legumes are Allen and Allen (1981) and Sprent (2001).

Other sources of nitrogen fixation in agroforestry systems

Apart from inputs from nitrogen-fixing trees, herbaceous cover crops/green manure legumes and grain legumes are often important components of agroforestry systems and further information can be found in Giller (2001). The discovery of endophytic nitrogen-fixing bacteria in the tissues (particularly the vascular tissues) of a range of non-legume species, including oil palm (*Elaeis guineensis*), has led to claims that such bacteria may give substantial inputs of nitrogen. Direct evidence for substantial nitrogen inputs (~20 kg N ha^{-1} $year^{-1}$) is strongest in sugar cane (*Saccharum officinarum*; e.g. Boddey, 1995), though the evidence is still controversial. Nitrogen inputs from heterotrophic, free-living nitrogen-fixing bacteria in soil are generally considered to be very limited and not of significance except over long time scales (Giller and Day, 1985; Vanderleyden, 1997; Giller, 2001).

Environmental limitations to nitrogen fixation

All environmental limitations that adversely affect plant growth and vigour also decrease amounts of nitrogen fixation in legumes, although the symbiosis is sometimes more sensitive to such constraints than other aspects of plant growth (Giller, 2001). Nitrogen fixation is sensitive to nutrient deficiencies, in particular phosphorus deficiency, which may restrict nodule formation if acute. Molybdenum deficiency influences nitrogen fixation directly as molybdenum is a component of the nitrogenase enzyme. Nitrogen fixation is thought to be more sensitive to drought stress than other processes, such as photosynthesis, although the evidence is somewhat equivocal (Sprent, 1984). A further interesting feature of the legume–rhizobium symbiosis is that the sensitivity to stress may be expressed through the bacteria, the legume host or the formation of the symbiosis itself. Legumes are invariably more sensitive to salinity than are rhizobia (Sprent, 1984), but rhizobia are more sensitive than their hosts to heavy metal pollution (Giller *et al.*, 1998). The infection process itself

appears to be particularly sensitive to calcium deficiency (Giller, 2001). Large differences in sensitivity to stresses such as aluminium toxicity in soil are found among rhizobial strains and among legume hosts.

Effects of management on nodulation and nitrogen fixation

Legume trees are often pruned severely in agroforestry systems to provide fodder or foliage for soil amendment. It is well established that defoliation causes nodule senescence (Witty and Minchin, 1988), and pruning or browsing of trees by animals causes temporary decreases in the rates of nitrogen fixation. Reestablishment of nitrogen fixation will depend on formation of new nodules, though this can sometimes be rapid as legumes often harbour latent infections in young roots, which can develop when nitrogen demand in the plant is large. However, in *Erythrina poeppigiana* pruning resulted in complete mortality of nodules and there was a lag of 10 weeks before active nodules were re-formed (Nygren and Ramírez, 1995). Similarly, pruning of *Leucaena diversifolia* resulted in drastic reduction of nodule activity (measured as acetylene reduction) for about 3 months (Snoeck, 1995). Nygren (1995) highlighted the danger that nodulation might be completely suppressed if trees are pruned too frequently.

Nodule senescence associated with defoliation of trees is a mechanism by which nitrogen is made available to plants growing in close proximity, though amounts of nitrogen made available in this way are likely to be relatively small (Rao and Giller, 1993; Nygren and Ramírez, 1995). Even

Table 13.1. Mechanisms by which nitrogen from nitrogen-fixing trees can be made available to other plants.[a]

Mechanism	Rate of transfer	Likely importance as an N source
Below ground		
Root and nodule senescence and mineralization	Slow	Major
Rhizodeposition	Rapid	Minimal
Transfer between roots by interconnected mycorrhizal hyphae	Rapid	Minimal
Above ground		
Mineralization of severed or senesced plant material	Slow	Major
Consumption by grazing animals or insects and return in excreta or as carcasses	Slow/rapid	Major
Foliar leachates	Rapid	Moderate
Transfer of ammonia to associated plants	Rapid	Minimal

[a]Modified from Ledgard and Giller (1995).

Box 13.1. Rhizobia that nodulate legume trees.

The rhizobia that nodulate legume trees are a sadly neglected area of study compared with the amount of research effort which has been directed to grain legumes. The taxonomy of rhizobia has developed rapidly since the advent of phylogenetic methods for bacterial classification based on sequence analysis of the 16S rRNA gene (Young and Haukka, 1996). The older classification methods were largely based on the legume host range of the rhizobia. Although this approach was recognized to be severely flawed early on (Wilson, 1944), the new classification reveals the true extent of such problems in that nodulation ability is closely related to the nodulation genes carried by the rhizobia, rather than their evolutionary similarity (Young and Haukka, 1996). The nodulation genes are generally carried on transmissible plasmids in fast-growing rhizobia, and in some cases on transmissible 'symbiotic islands' of chromosomal DNA (Sullivan and Ronson, 1998). Rhizobia far apart on the phylogenetic tree may thus carry the same nodulation genes and have a very similar host range for nodulation and nitrogen fixation.

Research in the last 10 years has revealed some surprising overlaps between rhizobia which can nodulate legume trees, herbaceous and grain legumes. The best documented case is that of *Rhizobium* sp. NGR234, which has been shown to effectively nodulate legumes from 112 genera, including members of the three different subfamilies of the *Leguminosae* (Pueppke and Broughton, 1999). Other broad-host-range rhizobia that nodulate trees are *Rhizobium tropici* (Martínez-Romero *et al*., 1991), which nodulates *Leucaena* spp. and *Phaseolus vulgaris*, and indeed other rhizobia species such as *R. etli*, which were thought to be specific in their host range, have since been shown to nodulate a wider range of species including trees (Hernandez Lucas *et al*., 1995).

Slow-growing rhizobia are all members of a single genus, *Bradyrhizobium*, whereas the fast-growing rhizobia have been split into several genera. Five genera of fast-growing rhizobia are currently recognized: *Rhizobium, Azorhizobium, Sinorhizobium, Mesorhizobium* and *Allorhizobium*, although this field is changing rapidly. Some trees are reported to nodulate only with slow-growing rhizobia (Dreyfus and Dommergues, 1981) but the vast majority examined nodulate with fast-growing rhizobia. Of particular note are the rhizobia which form stem nodules; shrubby legumes of the genus *Aeschynomene* form stem nodules with photosynthetic slow-growing *Bradyrhizobium* strains, whereas *Sesbania rostrata* forms nodules with rhizobia classified into a separate genus, *Azorhizobium caulinodans* (Giller, 2001).

Recent studies of rhizobia that nodulate legume trees have resulted in the description of the new species *Sinorhizobium terangae* and *Sinorhizobium saheli* from nodules of *Sesbania* and *Acacia* spp. in Senegal (de Lajudie *et al*., 1994) and *Mesorhizobium plurifarium* (de Lajudie *et al*., 1998), *Sinorhizobium arboris* and *Sinorhizobium kostiense* (Nick *et al*., 1999) from nodules of *Prosopis* and *Acacia* spp. in Sudan and Kenya. The classification has recently been substantially revised (see Young *et al*., 2001)

with *E. poeppigiana*, which was profusely nodulated in the field, amounts of nitrogen released from complete senescence of the nodules were much less than 10 kg N ha^{-1} (Nygren and Ramírez, 1995). If the turnover of fine roots is included, the amounts of N recycled below ground are likely to be of considerable importance but accurate estimates are lacking. The major pathway by which nitrogen is made available for other plants is through recycling of above-ground litter either directly to the soil, or through manure of animals grazing on the trees (Table 13.1).

Requirements for inoculation

The success of many of the fast-growing legume trees in agroforestry has undoubtedly been due to their ability to nodulate and fix nitrogen in soils across the tropics. Unfortunately, it is almost impossible to generalize as to which species are likely to nodulate readily in soils where they have not been grown, although there are gradations of the degree of promiscuity or specificity among agroforestry trees (Bala and Giller, 2001). Bala (1999) hypothesized that the success of species such as *Leucaena leucocephala* and *Gliricidia sepium* could have been due to either: (i) their ability to nodulate promiscuously with a wide range of rhizobial types, or (ii) the fact that, although such trees may nodulate with a narrow range of rhizobial species, these species are ubiquitous throughout the tropics. To test these hypotheses he characterized rhizobial isolates from nodules of *Leucaena leucocephala*, *Calliandra calothyrsus*, *Gliricidia sepium* and *Sesbania sesban* grown in soils from Central and South America, West, East and southern Africa and South-east Asia. Rhizobial isolates were characterized on the basis of physiological tests, host range for nodulation and molecular typing methods (including 16S rRNA partial gene sequences). Bala (1999) found that rhizobial isolates nodulating *Leucaena*, *Calliandra* and *Gliricidia* were diverse, belonging to a wide range of strains of the genera *Rhizobium*, *Mesorhizobium*, *Sinorhizobium* and *Allorhizobium/Agrobacterium*. This supports the first hypothesis that the tree legumes nodulated promiscuously with a wide range of rhizobia. Further, these three tree legumes nodulated with many of the same isolates although there were isolates which nodulated with only one or two of the species. *Sesbania sesban* was an exception in that it nodulated with a largely separate range of isolates, which fell into the *Rhizobium* and *Mesorhizobium* genera. Even more surprising was the limited range of soils from southern Africa in which *S. sesban* nodulated, considering that it is naturally found in this region. This clearly indicated that *S. sesban* requires inoculation when sown for the first time into upland soils. Details of how rhizobial inoculants can be made and applied are given in the methods manuals cited below.

13.2 Microbiological Methods for Studying Rhizobia

Methods for the isolation of rhizobia from nodules and authentication are simple and can be conducted in any laboratory which has the capacity for simple microbiology. There are a number of excellent methods manuals including Vincent (1970), Somesagaran and Hoben (1985) and Sylvester-Bradley and Kipe-Nolt (1988). Host-range studies provide information on the ability of individual isolates to nodulate and fix nitrogen with different legumes, and therefore can give some clues as to the phylogenetic relatedness of strains, but detailed strain characterization now relies on methods of molecular biology, which tends to restrict such activities.

13.3 Simple Methods for Determining Whether a Legume is Fixing Nitrogen

In the absence of access to isotope-based methods (see below) there are some simple approaches to assessing whether trees or crops are benefiting from nitrogen fixation.

- The simplest and most obvious (though often overlooked) approach to determine whether a tree is fixing nitrogen is to excavate the root system and look for nodules. If nodules are found, then this is strong evidence that the trees are or have recently been fixing nitrogen. If no nodules are found this could be due to a number of reasons: that the root excavations are not complete and the nodules are only found in deeper horizons, or that the nodules readily become detached from the roots and lost during sampling, which is particularly problematic with some species (e.g. *Cajanus cajan, Gliricidia sepium*).
- The ability of a tree to grow and accumulate nitrogen in infertile or skeletal soils may be evidence of nitrogen fixation. Sanginga *et al.* (1986) compared nitrogen accumulation in prunings of inoculated and uninoculated *Leucaena leucocephala* and estimated that 224–274 kg N ha^{-1} came from nitrogen fixation. This approach to estimating nitrogen fixation, which is a simple and useful approach to estimating nitrogen benefits, is referred to as the nitrogen balance method. However, in many situations it is dangerous to rely on such evidence alone as tree roots might be accessing deep soil horizons richer in nitrogen (see Box 8.1 on p. 171), or there may be subsurface lateral flow of water from which the trees can scavenge nitrogen. A further development of this approach is to develop a whole nitrogen balance for the system, preferably comparing a system with nitrogen-fixing trees with a system with non-nitrogen-fixing trees. Such studies suffer from the large number of unknown processes such as gaseous losses,

which are as difficult to estimate as nitrogen fixation. A primary advantage of agroforestry systems is that the trees are able to access nutrient sources which are not available to other plants, which in itself can cause problems in calculations of nitrogen balances if nitrogen is taken up from deep water tables.

13.4 Isotope-based Methods for Measurement of Nitrogen Fixation

^{15}N-isotope-based methods are perhaps the only means available for estimating amounts of nitrogen from nitrogen fixation in trees growing in the field, though such methods may have significant problems in their application. The two main variants of these methods are those involving addition of ^{15}N-labelled fertilizers (often called ^{15}N-isotope dilution methods) and those that rely on the natural ^{15}N-enrichments of soil (known as natural abundance methods). Most soils are naturally, slightly enriched with ^{15}N with δ^{15}N values commonly of 2–16‰ (the difference between the ^{15}N content of the material and air expressed in parts per 1000):

$$\delta^{15}\text{N (in parts per 1000 or } {}^{0}/_{00}) = \left(\frac{R_{\text{sample}} - R_{\text{reference}}}{R_{\text{reference}}} \right) \times 1000 \qquad (13.1)$$

where during chemical analysis R is the ratio of N_2 molecules derived from the plant material, which are composed of one ^{15}N and one ^{14}N atom to those composed of two ^{14}N atoms, i.e.

$$R = \frac{^{15}\text{N} + {}^{14}\text{N}}{^{14}\text{N} + {}^{14}\text{N}} \qquad (13.2)$$

The basic principle of both methods is that nitrogen taken up from the soil is enriched with the heavy isotope ^{15}N compared with nitrogen absorbed from the atmosphere. A non-nitrogen-fixing reference plant is used to estimate the ^{15}N-enrichment of nitrogen available from the soil in both cases (Peoples *et al.*, 1989). The major assumption (and problem) of these methods is that the ^{15}N-enrichment of the available soil nitrogen taken up by the reference plant is the same as that taken up by the nitrogen-fixing legume. This is in fact only true when there is a perfectly uniform ^{15}N-enrichment of plant available nitrogen in the soil with depth and time, or, if this is not the case, when the rooting depths and activity and the time course of nitrogen uptake of the reference and legume plants are identical (Witty, 1983). This assumption is rarely satisfied and therefore steps must be taken to limit the variability in ^{15}N-enrichment of the available soil nitrogen as far as possible (Witty and Giller, 1991). The use of direct measurements of the ^{15}N-enrichment of the available soil nitrogen

coupled with modelling have been recommended as an alternative to using reference plants, but such approaches require extensive sampling and analysis of extractable soil nitrogen (Chalk and Ladha, 1999) and are unlikely to be widely used. Much has been written on the problems of measuring nitrogen fixation using isotope approaches (e.g. Witty, 1983; Witty and Giller, 1991; Chalk and Ladha, 1999). Problems encountered with herbaceous legumes when isotope methods are applied are magnified in studies with trees due to their much larger size and longevity (Boddey *et al.*, 2000). The size of trees and lignification of plant parts presents additional problems of subsampling of plant tissues to give a representative sample for measurement of tissue nitrogen concentrations and ^{15}N-abundance.

Box 13.2. Measuring nitrogen fixation under controlled conditions.

There are remarkably few measurements of nitrogen fixation by nitrogen-fixing trees growing in the field. This is in no small part due to the technical problems that exist in the application of the available methods, and partly for this reason many measurements have been confined to studies under more controlled conditions with trees grown in pots. The major limitations of these pot studies is that they do not often provide the information we need to assess the role of nitrogen fixation in agroforestry systems.

So what do we want to measure? Do we want to know which of the different provenances of *Gliricidia sepium* fix the most nitrogen after 6 weeks of growth in a glasshouse? Or do we wish to pose the question: are all rhizobial strains that belong to different species or genera equally efficient in nitrogen fixation with a particular tree species? If these are the types of questions raised, then experiments in pots are a valid approach, but we must further question what methods are required to address these questions.

The principal reason for using the ^{15}N-isotope dilution method is to distinguish between nitrogen derived from the atmosphere and nitrogen derived from the soil. If experiments are conducted in pots, the supply of nitrogen from the soil can be carefully controlled, so there is little advantage in using the ^{15}N-isotope dilution method compared with simpler nitrogen balance methods.

The major concern with such studies under controlled conditions in pots is that there is no guarantee that there is any correlation whatsoever between results found under controlled conditions in the glasshouse and the response of the same treatments in the field. This is largely due to the strong interactions between nodulation and nitrogen fixation and a wide range of environmental factors, which are described above.

13.5 Estimating Nitrogen Fixation in Field Settings

It can be argued that ^{15}N-isotope dilution using labelled fertilizers has no role to play in estimation of nitrogen fixation by trees, for the following reasons.

- A wide variability in rooting patterns can be found even within a single provenance of a tree species, as soil chemical and physical properties exert a very strong influence on root distributions. As mentioned before it is difficult to ensure that ^{15}N is incorporated to depth and unlikely that non-fixing plants can be identified that have matching rooting patterns.
- One of the major advantages of legume trees is that they can be deep-rooting, but this raises obvious problems in uniform ^{15}N-labelling of soil with depth. These are problems that also apply to measurement of nitrogen fixation in herbaceous legumes but are amplified in agroforestry systems because of the larger size and potentially deeper-rooting systems of the trees.
- ^{15}N-isotopes are expensive. Although it can be argued that the cost of the isotopes is relatively small compared with other costs of field experimentation and mass spectrometric analysis of samples, the wide planting arrangements of trees in agroforestry systems demand the use of large ^{15}N-labelled microplots. There is, therefore, a strong temptation to try to cut costs by not including border rows in the ^{15}N-labelled microplots, but sampling the whole area to which ^{15}N-labelled fertilizers have been applied. Obviously, this breaks the basic rules of experimentation with ^{15}N-labelling (see, for example, Stumpe *et al.*, 1989). Given the extensive rooting systems which have often been observed in agroforestry trees, border rows are particularly important (see Section 3.2).

This means that the ^{15}N natural abundance method is probably the only sensible choice for measuring nitrogen fixation by trees in the field, but again problems should not be underestimated. The $\delta^{15}N$ of soil nitrogen may vary markedly with depth, although the extent to which this is a problem is debatable (Boddey *et al.*, 2000). Some studies have found little change of $\delta^{15}N$ in the plant-available nitrogen with depth, but there is considerable variation between sites so this should be investigated if possible. It is unlikely that a reference tree can be identified which will have an identical pattern of root activity with depth, or uptake with time, as that of the nitrogen-fixing tree under investigation. One approach to this problem is to use a range of potential reference plants. This was recommended for measurements of nitrogen fixation in grain legumes (Boddey *et al.*, 1990) but is especially applicable for nitrogen-fixing trees. If most of the reference plants give $\delta^{15}N$ values close to the $\delta^{15}N$ of the total

Kjeldahl nitrogen of the soil on which they are growing, but one reference plant consistently gives $\delta^{15}N$ values significantly different from the rest, then that is a reasonable indication that it is an aberrant reference value and may be discarded. Such a situation was found when measuring nitrogen fixation in *Faidherbia albida* in Malawi where mango (*Mangifera indica*) consistently gave small $\delta^{15}N$ values (2.7–4.8‰) compared with the soils (6.4–11.5‰) and other non-fixing trees on the same sites (6.0–10.0‰) (Phombeya, 1999).

The reason(s) why certain plants tend to become depleted or enriched in ^{15}N relative to their sources of nitrogen are not fully understood but appear to be related to mycorrhizal colonization, drought and nitrogen deficiency (Högberg and Alexander, 1995; Handley *et al.*, 1996, 1999). Högberg (1990) showed that ectomycorrhizal non-nodulating legume trees (*Brachystegia* and *Julbernardia* spp.) in miombo woodland of Tanzania were more depleted in ^{15}N than arbuscular mycorrhizal-nodulating or non-nodulating legume trees. Subsequent studies in Cameroon did not confirm that these differences were due to the type of mycorrhizal infection (Högberg and Alexander, 1995). Other research suggests that $\delta^{15}N$ depletion in trees may be related to fractionation during transfer from mycorrhizas and depletion of $\delta^{15}N$ in tree shoots may indeed be indicative of mycorrhizal status (Hobbie *et al.*, 1999, 2000). Problems of this kind must be borne in mind if the $\delta^{15}N$ method is to be used. As a general rule, other indicators of nitrogen fixation such as nitrogen accumulation and the ability of the tree to nodulate should be ascertained before this method is used. Application of the ^{15}N natural abundance method for measuring nitrogen fixation by trees is reviewed in detail by Boddey *et al.* (2000).

13.6 Methods Based on Nitrogen Fixation Transport Products

Nitrogen is taken up from soil principally in the form of nitrate, whereas fixed nitrogen is assimilated into amides or ureides depending on the host legume before transport to the shoots. The proportion of nitrogen derived from nitrogen fixation at any given time can be estimated based on the relative proportion of the nitrogen in the xylem sap present as ureides (Peoples *et al.*, 1989). Samples of bleeding sap from cut shoots are taken for analysis of total nitrogen and ureide nitrogen. This method can also be applied by extraction of dried petioles and determination of the ureide content, which makes sampling easier (Peoples *et al.*, 1989).

Ureide production appears to be limited to legumes of the tribes *Phaseoleae, Indigoferae* and *Desmodieae* (Giller, 2001), so that the only agroforestry species confirmed to transport ureides are *Cordariocalyx gyroides* and *Desmodium rensonii* (Herridge *et al.*, 1996). *Flemingia macrophylla* belongs to the tribe *Phaseoleae* and may therefore transport ureides (Giller,

2001), but this has yet to be confirmed. Comparisons of field estimates of nitrogen fixation in *Cordariocalyx* with the ^{15}N natural abundance method showed close correspondence with estimates made using the ureide method (Peoples *et al.*, 1996). Reports of ureide production in species within the tribe *Robineae* (such as *Sesbania* and *Gliricidia*) and the *Mimoseae* are almost certainly due to artefacts produced by coloured compounds during the analysis procedure. Attempts have been made to estimate nitrogen fixation in amide-exporting legumes using xylem sap analysis, but with less success (Peoples *et al.*, 1989).

13.7 Estimating Total Amounts of Nitrogen Fixation

With all of the above methods, biomass estimates and tissue nitrogen analyses are required to calculate actual amounts of nitrogen fixation, using these estimates of %N from nitrogen fixation. Although it can be seen that estimation of nitrogen fixation is difficult, there is still remarkably little quantitative information given the potential importance of nitrogen fixation in the long-term sustainability of agroforestry systems.

Chapter 14
Mycorrhizas

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14.1 Synopsis

Most trees and crop plants form mycorrhizal associations but our understanding of their role, particularly in mixed species associations, is still developing. Whereas trees form associations with both ectomycorrhizal (Fig. 14.1) and arbuscular mycorrhizal (or endomycorrhizal) fungi (see Fig. 15.4 on p. 295), herbaceous crop plants only form arbuscular mycorrhizas. There is strong evidence to show that mycorrhizal colonization can be of great benefit to plants but that effects of colonization vary markedly depending on environmental conditions and species of both host plant and fungus. The benefits to host plants are assumed to be



Fig. 14.1. *Pinus strobus* root with ectomycorrhizal colonization.

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because mycorrhizal colonization may improve nutrient uptake, water relations, resistance to soil pathogens, and resistance more generally to adverse soil conditions. Furthermore, since mycorrhizas may infect many different host species, the mycelial network they develop below ground may connect plants and facilitate transfers of carbon and nutrients among them. In this chapter, the evidence for potential effects of mycorrhizal colonization is discussed, leading to the identification of key research questions and then description of the methods required to address them.

Importance of mycorrhizas for plant nutrition

The improved mineral nutrition of mycorrhizal plants is well documented (Marschner, 1995). In particular, a role in the uptake of phosphorus by ectomycorrhizas or arbuscular mycorrhizas, and nitrogen uptake by ectomycorrhizas and to a lesser extent arbuscular mycorrhizas, has been described (George *et al.*, 1995). In *Eucalyptus grandis* and *Eucalyptus maculata*, inoculation with the ectomycorrhizal fungus *Pisolithus* sp. enabled the seedlings to utilize organic nitrogen sources, which could not be utilized by non-mycorrhizal seedlings (Turnbull *et al.*, 1995). In addition to the elements phosphorus and nitrogen, mycorrhizas have been shown to facilitate plant acquisition of magnesium (Jentschke *et al.*, 2000), copper and zinc (Guo *et al.*, 1996) and manganese (Marschner, 1995). The effects of arbuscular mycorrhizas on mineral acquisition of plants have recently been reviewed by Clark and Zeto (2000).

Both ectomycorrhizas and arbuscular mycorrhizas can greatly increase the volume of soil exploited due to the extent and high surface area of the extramatrical mycelium. It has been shown in a number of investigations that the external hyphae of mycorrhizas can absorb phosphorus from outside the root depletion zone and transport it to the host plant. Some of the most convincing demonstrations of this have used experimental designs with separate root and hyphal compartments. In these investigations roots were excluded from the hyphal compartment by a 30 μm net which allowed mycorrhizal hyphae to pass. With such designs, in white clover (*Trifolium repens*) colonized by arbuscular mycorrhizas, a depletion zone of more than 11 cm due to phosphorus uptake by the extramatrical hyphae could be shown (Li *et al.*, 1991). In an investigation with Norway spruce (*Picea abies*) and the ectomycorrhizal fungus *Paxillus involutus*, using a separate root and hyphal compartment, translocation of phosphorus by the extramatrical mycelium over a distance of 5 cm was demonstrated (Brandes *et al.*, 1998). In *Pinus sylvestris* mycorrhizal with *Suillus bovinus*, phosphorus was translocated over 30 cm, mainly in rhizomorphs (Finlay and Read, 1986a). Effective uptake of phosphorus by the hyphae of both ectomycorrhizas and arbuscular mycorrhizas may also

be because polyphosphates are accumulated in vacuoles, where they act as both a storage form of phosphorus and function in energy storage.

In addition to the increase in surface area provided by the extramatrical mycelium, ectomycorrhizas have also been shown to exude organic acids and mobilize mineral phosphorus from sparingly soluble sources. The involvement of organic acid exudation in uptake of phosphorus in non-mycorrhizal roots is well documented (Marschner, 1995). The ectomycorrhizal fungus *Paxillus involutus* exudes high amounts of malate and oxalate (Lapeyrie *et al.*, 1987; Marschner, 1994). Cumming and Weinstein (1990) demonstrated that *Pinus rigida* ectomycorrhizal with *Pisolithus tinctorius* was able to extract phosphorus from insoluble aluminium phosphate. Recently, it has been suggested that ectomycorrhizas are a primary factor in the weathering of minerals (Jongmans *et al.*, 1997; Landeweert *et al.*, 2001). Thus there is a strong possibility that organic acids are involved in the uptake of phosphorus and also other nutrients such as potassium, calcium and magnesium (Landeweert *et al.*, 2001) by ectomycorrhizas.

Both ectomycorrhizas and arbuscular mycorrhizas have high levels of phosphatase activity. In wheat (*Triticum aestivum*), roots infected with arbuscular mycorrhiza had higher levels of acid phosphatase than non-mycorrhizal roots (Tarafdar and Marschner, 1994). In Norway spruce ectomycorrhizal with a number of fungi, similar levels of acid phosphatase were found in mycorrhizal and non-mycorrhizal roots (Eltrop, 1993). However, in Norway spruce mycorrhizal with *Thelephora terrestris*, a higher phosphomonoesterase activity was found in mycorrhizal roots and rhizomorphs than in non-mycorrhizal roots. These results indicate that mycorrhizas have a high potential to mobilize organic phosphorus, but that there are differences between species of mycorrhizas. However, even if the levels of acid phosphatase activity are similar between mycorrhizas and non-mycorrhizal roots, the large surface area of the extramatrical mycelium will greatly increase the potential to mobilize organic phosphorus.

There are few investigations of the contribution of mycorrhizas to the total uptake of phosphorus by a plant. In Norway spruce ectomycorrhizal with *Paxillus involutus*, 52% of the total phosphorus uptake was shown to be via the extramatrical hyphae (Brandes *et al.*, 1998). In arbuscular mycorrhizal *Trifolium repens*, the hyphae contributed 70–80% of the total phosphorus uptake (Li *et al.*, 1991). In a similar system with *Trifolium repens*, phosphorus uptake in non-mycorrhizal plants was only 3% of the uptake of mycorrhizal plants (Schweiger *et al.*, 1999). In non-mycorrhizal *Leucaena leucocephala*, soil solution phosphorus concentrations 27–38 times higher were required for maximal growth than in mycorrhizal seedlings (Habte and Manjunath, 1987). These data indicate that mycorrhizas have a high potential to contribute to phosphorus acquisition by plants. It is

possible that experimental systems overestimate the mycorrhizal contribution to phosphorus acquisition but, since the mycorrhizal contribution measured experimentally is so high, it is likely that under field conditions mycorrhizas play a very significant role in phosphorus acquisition.

Importance of mycorrhizas for plant water relations

Both ectomycorrhizas (Lamhamedi *et al.*, 1992) and arbuscular mycorrhizas (Allen, 1991) can improve the water relations of plants. For example, in two species of *Acacia*, Cornet and Diem (1982) showed that mycorrhizal plants had a higher number of open stomata under drought conditions. However, the mechanisms involved are still controversial. A number of mechanisms of increased host drought tolerance have been suggested, such as increased root hydraulic conductivity (Koide, 1985), alteration of stomatal regulation due to hormone signals (Allen *et al.*, 1982; Barea and Azcón-Aguilar, 1982), osmotic adjustment (Augé *et al.*, 1986), hyphal water transport (Hardie, 1985; Faber *et al.*, 1991; Ruiz Lozano and Azcón, 1995) and improved phosphorus nutrition (Fitter, 1988).

Improved water status has often been associated with improved host phosphorus nutrition (Nelsen and Safir, 1982; Graham and Sylvertson, 1984; Fitter, 1988). Mycorrhization nearly always results in an improved phosphorus nutrition and/or larger size of host plants, which often makes it difficult to deduce the mechanisms involved in improved water relations. However, a number of authors have shown that host water status is improved independently of host phosphorus nutrition (Augé *et al.*, 1986; Bethlenfalvay *et al.*, 1988; Peña *et al.*, 1988; Sanchez-Diaz *et al.*, 1990). Davies *et al.* (1992) increased the phosphorus nutrition of non-mycorrhizal plants by addition of fertilizers to produce pepper (*Capsicum annum*) plants mycorrhizal with *Glomus deserticola* and non-mycorrhizal pepper plants with similar levels of phosphorus. After drought acclimation, mycorrhizal plants were the most drought tolerant. These authors suggested that the extraradical hyphae might facilitate water uptake and improve drought resistance. Faber *et al.* (1991) have suggested a role of the hyphae in the transport of water. These authors suggested that hyphae of arbuscular mycorrhizas might have access to smaller pores within the soil and facilitate water uptake under drought conditions.

To estimate the contribution of the extraradical hyphae to water uptake, techniques have been used with separate hyphal and root compartments, divided by a nylon screen. Using such techniques, George *et al.* (1992) found no evidence for the involvement of hyphae of arbuscular mycorrhizas in water transport of *Agropyron repens*. Using a similar experimental design, Ruiz Lozano and Azcón (1995) were able to show

that, in association with lettuce (*Lactuca sativa*), extraradical hyphae of both *Glomus deserticola* and *Glomus fasciculatum* were involved in water uptake. However, differences in water uptake were shown for the two fungi under different water regimes. Under poor water supply *Glomus deserticola* transported a larger amount of water than *Glomus fasciculatum*, suggesting species differences in hyphal water uptake and transport.

Effects of mycorrhizas on plant–pathogen interactions

Apart from beneficial effects on the nutrition and water supply of host trees, mycorrhizas have been proven to increase the resistance of trees to infection by pathogens of the fine roots (Marx, 1972; Duchesne *et al.*, 1989). Different species of mycorrhizal fungi vary in their efficiency at preventing root infections, although the mechanisms involved in this process are rather poorly understood. Possible modes of action include the production of antibiotics by the mycorrhizal fungi, stimulation of host defence mechanisms and the physical barrier presented by the Hartig net in ectomycorrhizal roots (Marx, 1972). Gunjal and Patil (1992) showed that arbuscular mycorrhizal fungi increased the resistance of casuarina (*Casuarina equisetifolia*) to *Fusarium vesicubesum*. Infections of wheat with common root rot (*Bipolaris sorokiniana*) and root disease caused by *Gaeumannomyces graminis* and *Fusarium culmorum* were reduced by arbuscular mycorrhizal fungi (Thompson and Wildermuth, 1989; Innocenti and Branzanti, 1998). In the studies by Innocenti and Branzanti (1998), the colonization of the roots with *Glomus* did not reduce the disease index, but increased host tolerance to pathogen attack. Both ectomycorrhizal and arbuscular mycorrhizal fungi have been shown to produce antibiotics (Marx, 1969; Trofast and Wickberg, 1977). In addition, ectomycorrhizal fungi produce phenolic substances (Strobel and Sinclair, 1991) and oxalic acid (Duchesne *et al.*, 1989), which suppress root pathogens. Arbuscular mycorrhizal fungi have also been shown to reduce damage to plants by sedentary endoparasitic nematodes (Borowicz, 2001). An ectomycorrhizal fungus (*Pisolithus* sp.) increased the growth of nematode-sensitive *Acacia holosericea* seedlings under glasshouse conditions and reduced the multiplication of the root-knot nematode, *Meloidogyne javanica*, possibly through the production of polyphenolic compounds (Duponnois *et al.*, 2000).

Transfer of carbon and nutrients between plants

Ectomycorrhizas and arbuscular mycorrhizas form a large mycelia network in the soil. As trees of different species may be colonized by the same

species of ectomycorrhiza, and arbuscular mycorrhizas mostly have a wide host range, different species of trees and other plants may be connected by a common below-ground hyphal network. It has often been suggested that this hyphal network of mycorrhizas forms a pathway for the movement of carbon and nutrients between plants (Finlay and Read, 1986b). In forests with ectomycorrhizal trees a movement of carbon between trees has been shown. In laboratory studies, transfer of carbon has also been demonstrated between plants connected by an arbuscular mycorrhizal mycelium (Francis and Read, 1984).

Although the transfer of carbon between species or between mature trees and seedlings of a species is of great ecological interest, in agroforestry and agricultural systems the transfer of nutrients, especially nitrogen and phosphorus, is more important. This is particularly the case for the transfer of nitrogen from nitrogen-fixing trees or crops to receiver plants. This invariably requires the movement of nutrients between arbuscular mycorrhizal plants. There are conflicting reports about the role of mycorrhizas in the transfer of nitrogen between plants (Hamel *et al.*, 1991; Frey and Schüepp, 1993). Frey and Schüepp (1993) showed that the mycorrhizal mycelium enhanced the transfer of nitrogen between clover (*Trifolium alexandrinum*) and maize (*Zea mays*). In contrast, Hamel *et al.* (1991) found that the transfer between soybean (*Glycine max*) and maize was small. The transfer increased if there was direct contact between the roots. Between soybean and maize a greater transfer occurred for nitrogen supplied from fertilizer than nitrogen from biological fixation (Marschner, 1995). This suggests that plants restrict the movement of symbiotically fixed nitrogen. There is little evidence to show that there is a transfer of nitrogen from the plant to the fungus (Ikram *et al.*, 1994).

The direct transfer of nitrogen between plants may, however, be a function of the sink–source relationship between potential donor and receiver plants (Frey and Schüepp, 1993). Ekblad and Huss-Danell (1995) showed that in a mixture of *Alnus incana* and *Pinus sylvestris* the proportion of fixed nitrogen in *P. sylvestris* derived from *A. incana* was highest (9%) when *P. sylvestris* was nitrogen starved. Ikram *et al.* (1994) investigated nitrogen movement between *Pueraria phaseoloides* as a nitrogen-fixing donor plant and rubber trees (*Hevea brasiliensis*) as receiver plants. *P. phaseoloides* is used as a cover crop in rubber plantations. In the investigation, the shoot of the donor plant was shaded or removed to increase the difference in sink strength in favour of the receiver plant. Despite this treatment *P. phaseoloides* only supplied 0.07–0.4% of the total nitrogen uptake of *H. brasiliensis*. This suggests that mycorrhizal links play only a small role in the transfer of nitrogen in this system. However, it is known that intercropping of rubber with nitrogen-fixing *P. phaseoloides*, as in other intercropping systems, increases the growth of rubber (Broughton, 1977). The roots and mycorrhizas may take up nitrogen

released by the decomposition of leaf and root litter. It has also been suggested that nitrogen may be transferred via root exudation of nitrogen compounds (see Table 13.1 on p. 262). Transfer from the roots may also be higher if the roots of the donor plants are dying. Johansen and Jensen (1996) found a significant transfer of nitrogen from pea (*Pisum sativum*) to barley (*Hordeum vulgare*) in mycorrhizal systems when, after removal of the shoot, the roots of the donor plant were dying. Mycorrhizas are able to take up and transport both inorganic and organic forms of nitrogen (George *et al.*, 1995), both of which may be available in dying roots. Such a transfer may be of particular significance after pruning of nitrogen-fixing trees in agroforestry systems, and in trees with high rates of root turnover.

Differences between mycorrhizal species and strains

In an investigation of the distribution of arbuscular and ectomycorrhizal tree species in savannas, Högberg (1989) suggested that ectomycorrhizal trees dominate soils with low levels of nitrogen and phosphorus, whereas arbuscular mycorrhizal species dominate soil low in phosphorus but relatively rich in nitrogen. This suggests that ectomycorrhizal trees may have a greater capacity for nitrogen acquisition than arbuscular mycorrhizal species. However, in the humid tropics of French Guiana, ectomycorrhizal tree species were found not to dominate the poorest soils (Bereau *et al.*, 1997).

The considerable benefits of mycorrhization can be seen in the advantages conferred by efficient species and strains. There are, however, significant differences between the effects that different species and strains of both ectomycorrhizas and arbuscular mycorrhizas have on host plants. It has been shown that the potential to take up phosphorus varies strongly between species both for ectomycorrhizas and arbuscular mycorrhizas (Marschner, 1995). As described above, species differences have also been demonstrated for improved water relations and pathogen resistance. Similarly, there are considerable functional differences between species of mycorrhizas and different species of trees. Bâ *et al.* (2000) demonstrated that only five species of tropical fruit trees (*Zizyphus mauritiana*, *Tamarindus indica*, *Dialium guineensis*, *Parkia biglobosa* and *Cordyla pinnata*) out of a total of 15 species had higher dry weight as a result of inoculation with *Glomus aggregatum*. In *Zizyphus mauritiana* and *Cordyla pinnata* a smaller positive effect was shown after inoculation with *Glomus intraradices*.

At present there is a potential danger of either underestimating or overestimating the significance of mycorrhizas because only a small number of mycorrhizal species have been investigated. For example, to reach reliable estimates of the contribution of mycorrhizas to phosphorus

uptake by plants in the field, we need to know the species composition of the mycorrhizas colonizing roots and have information about the phosphorus uptake capabilities of each species. The development of molecular biological methods of species identification for both ectomycorrhizas and arbuscular mycorrhizas has greatly increased our understanding of species composition (see Section 14.5).

Inoculation and management of mycorrhiza populations

Considering the advantages conferred by mycorrhizal infection, it is easy to understand why inoculation with mycorrhizas normally greatly increases growth and survival of host plants over that of non-mycorrhizal plants. Increased growth as a result of inoculation with mycorrhiza has been shown on numerous occasions in plants used in agroforestry systems (Osonubi *et al.*, 1991). However, the majority of studies that have investigated the advantages of mycorrhizal infection have compared mycorrhizal plants with non-mycorrhizal plants, which is unrealisitic since in most soils, with the exception of soils with an extremely low inoculation potential, most plants will form some sort of mycorrhizal association albeit weak.

However, with an increasing knowledge of the importance of mycorrhizal species differences, there is an increasing interest in the introduction of mycorrhizal species that are more efficient than the species present in a given soil under field conditions. Once introduced, these mycorrhizas must persist in the soil together with the indigenous mycorrhizas. Due to the difficulties in species identification of mycorrhizas, especially arbuscular mycorrhizas, there are few studies to estimate the persistence of introduced mycorrhizal species. With the development of better identification methods a clearer picture of mycorrhizal persistence will emerge. It is reasonable to assume that the highest success rate for introduced mycorrhizas will be achieved in soils with the least competitive indigenous mycorrhizal flora. However, even if introduced mycorrhizal species have a low persistence, the advantages conferred on the plant during establishment may be sufficient to justify their use. This may be particularly important for trees under adverse conditions, where establishment is often the biggest problem.

Management options for mycorrhizal populations in agricultural systems have been reviewed several times (Barea and Jeffries, 1995; Haselwandter and Bowen, 1996). Briefly these include using crop rotation techniques, precropping or intercropping. In each case a plant species is used to maintain a high inoculum potential in the soil. For example, a high level of mycorrhizal infection was maintained in cassava (*Manihot esculenta*) when it was grown in rotation with groundnut (*Arachis hypogaea*) (Sieverding and Leihner, 1984). Dodd *et al.* (1990a,b) showed that

precropping with cassava, kudzu (*Pueraria phaseoloides*) or sorghum (*Sorghum bicolor*) significantly increased arbuscular mycorrhizal infection of cowpea (*Vigna unguiculata*). As intercrops, millet (*Pennisetum glaucum*), Sudan grass (*Sorghum sudanense*) and sorghum increased the population densities of arbuscular mycorrhizal fungi and improved the growth of tree seedlings (Johnson and Pfleger, 1992). The reverse may also be true where, in agroforestry systems, highly mycorrhizal trees may be used to maintain the inoculum potential in the soil. Roots of *Citrus aurantifolia* grown in low-input systems had a higher degree of mycorrhizal colonization than those grown in conventional high-input systems in Mexico (Michel-Rosales and Valdés, 1996). These effects may have been related to fertilizer input, but the low-input systems were also structurally diverse, with a large number of other species including 16 species of fruit trees, compared with the high-input systems, which were monocultures. However, on a Nigerian Alfisol, Atayese *et al.* (1993) found that *Senna* sp., *Gliricidia* sp. and *Leucaena* sp. did not increase the mycorrhizal spore density of the soil even though *Gliricidia* sp. and *Leucaena* sp. were highly mycorrhizal. The mycorrhizal spore density was, however, increased by planting cassava (*Manihot esculenta*).

Research needs

Many of the studies cited above have been carried out under laboratory conditions. Therefore, there is a general requirement to transfer results obtained in laboratory experiments to the field. Many of the questions that need to be answered for mycorrhizas in tree–crop associations, listed below, are similar to the questions relevant for conventional agriculture and forestry.

- Considerably more information is required about the ecophysiology and effectiveness of different mycorrhizal species, as well as their below-ground population dynamics. Information about species should include estimates of which nutrient pools mycorrhizas are accessing and whether these differ from non-mycorrhizal plants.
- Information is also required about the persistence of introduced mycorrhizas, and whether persistence can be increased by cultivation techniques. Additionally, information is required on how management actions, such as mulching, crop rotation and fertilization (both mineral and organic), affect the development of mycorrhizas.

In addition there are some research questions specific to tree–crop associations.

- Can the association of crops with strongly mycorrhizal trees or tree species with particularly efficient mycorrhizas increase the access of these crops to less available nutrient pools in the soil via nutrient release from tree litter?
- Can strongly mycorrhizal species aid the establishment of poorly mycorrhizal species under adverse site conditions, for example in rehabilition of degraded lands?

14.2 Inoculation Methods

Propagules of mycorrhizal fungi include soil hyphae, spores, old root fragments and colonized particles of organic matter (Brundrett and Abbott, 1994). In particular, soil hyphae are important propagules under drought conditions. Soil disturbance and degradation often result in low inoculation potential of the soil; hence there is often a need to reintroduce mycorrhizal fungi in general or to introduce efficient mycorrhizal strains or species. Land management practices can then be used to maintain introduced mycorrhizas or enhance indigenous populations.

The simplest source of mycorrhizal propagules is soil from areas of high mycorrhizal activity. Soil may be used as a non-specific inoculum for both ectomycorrhizal and arbuscular mycorrhizal fungi. However, for reasons of phytosanitation it is often undesirable to use soil as inoculum. For this reason and also to allow introduction of specific fungi, artificial inocula are often required.

For ectomycorrhizas, inoculation techniques have been developed which use spores of selected fungi (Marx *et al.*, 1984). Mycelium of ectomycorrhizal fungi can easily be produced in fermenters. The mycelium can then be trapped in alginate beads for use as inoculum (Mauperin *et al.*, 1987).

Arbuscular mycorrhizal fungi cannot be cultured *ex planta* and thus must be cultured with a host plant. Most inoculum production requires growth of the host plants in a medium that can be used as a carrier for spores and hyphae. Carrier substances include expanded clays (Feldmann and Idczak, 1994) or vermiculite or perlite (Bagyaraj, 1994). Soil-free inoculum has been produced in aeroponic (Jarstfer and Sylvia, 1995) and nutrient-film cultures (Elmes and Mosse, 1984). The suitability of these inocula for different applications depends on the identity of the main mycorrhizal propagules and their ability to retain infectivity during storage and to persist in the soil from year to year, as well as on methods available for application (Smith and Read, 1997). Due to the expense of the production of inoculum and the bulk required, large-scale inoculation is often not a viable option. Thus management of indigenous populations is of great importance (see Section 14.1).

14.3 Sampling of Plant Roots for Mycorrhizal Studies

Collection of plant roots for estimating mycorrhizas requires sampling, preparation and storage of the roots. Root samples are normally collected using a trowel or soil auger (see Section 12.2). Roots are normally removed from the soil using careful washing with water or removal of the soil using forceps. Particularly for visual identification of ectomycorrhizas damage to the roots should be avoided. For quantification and identification (both visual and molecular) of arbuscular mycorrhizas and for molecular identification of ectomycorrhizas, roots can be stored in 50% (v/v) ethanol. For visual quantification and identification of ectomycorrhizas, roots can be stored in moistened Petri dishes at 4°C for several days. Fixation of ectomycorrhizas often results in colour changes, making visual identification more difficult.

14.4 Quantification of Mycorrhizas

Non-vital staining of arbuscular mycorrhizas

In order to visualize and quantify root colonization by arbuscular mycorrhizal fungi the fungal structures must be stained. The most commonly utilized stains are non-vital stains, which cannot distinguish between living and dead fungal tissues. Stains for light microscopy include trypan blue (Koske and Gemma, 1989) and chlorazol black E (Brundrett *et al.*, 1984). Acid fuchsin is also a popular non-vital stain; this stain has fluorescent properties, which are best visualized by fluorescence microscopy (Merryweather and Fitter, 1991).

One of the most frequently cited non-vital staining procedures utilizes the fungal staining properties of trypan blue (Phillips and Hayman, 1970; Koske and Gemma, 1989). Roots are washed from the soil and either stained directly or fixed in 50% ethanol and stained at a later time. The root tissue is cleared in 2.5% KOH at 90°C for 10–30 min in a water bath. After clearing, dark tissues may need to be bleached in a freshly prepared solution of alkaline hydrogen peroxide for 10–45 min. Following the KOH and bleaching steps, the root tissue is highly alkaline and requires acidification prior to staining with trypan blue. The roots are rinsed thoroughly and acidified in 1% HCl for 1–24 h (at least 8 h is generally recommended). The acidified roots can then be stained in 0.05% trypan blue in acidic glycerol at 90°C for 15–60 min. The stained tissues are then de-stained in acidic glycerol at room temperature. De-staining overnight generally improves the contrast between the fungal and root tissues, which often initially retain excess stain. Stained tissues can be stored in acidic glycerol in the dark for

many months. For the protocol details in full see Koske and Gemma (1989).

Visual methods of evaluation of arbuscular mycorrhizas

The assessment of fungal colonization is generally based on estimates of the proportion of potential host tissue (the root cortex) colonized by arbuscular mycorrhizal fungi (Giovannetti and Mosse, 1980; McGonigle *et al.*, 1990). The most extensively used visual method of determining fungal colonization is the grid-line intersect method (Giovannetti and Mosse, 1980; McGonigle *et al.*, 1990). This method is used to estimate the proportion of colonized tissue and total root length. A root sample is spread across a grid of known dimensions drawn on the base of a Petri dish. Using a dissecting microscope at ×40 magnification, each point at which a root intersects a vertical or horizontal grid line is studied and the presence or absence of fungal tissue is noted.

However, at ×40 magnification it is difficult to distinguish between different fungal structures. In light of this McGonigle *et al.* (1990) devised an objective method of measuring root colonization called the magnified intersection method. This method is used to obtain the percentage colonization of different fungal structures in a root system. Root sections, approximately 1 cm in length, are mounted on microscope slides in a few drops of acidic glycerol and viewed with a compound microscope at magnification ×200. Root/eye-piece cross-hair intersections are considered along the axis of the root section and the presence of arbuscules, hyphae and vesicles is noted at each intersection. A fixed number of intersections are considered per root section. From these data the percentage arbuscular, hyphal and vesicular colonization can be calculated.

Visual methods of evaluation of ectomycorhizas

The degree of colonization of ectomycorrhizas can be estimated by counting the number of mycorrhizal and non-mycorrhizal root tips in a representative sample (>100 root tips).

Vital staining

To determine the proportion of active fungal colonization associated with a root, vital staining procedures are employed. Vital stains locate sites of enzymatic activity, which are specific to viable fungal structures. Nitroblue

tetrazolium is a vital stain which is coupled to the activity of succinate dehydrogenase (a tricarboxylic acid enzyme), to produce formazan, which is purple (Smith and Dickson, 1991; Schaffer and Peterson, 1993). Used in conjunction with a non-vital stain such as acid fuchsin, the proportion of active to total colonization can be assessed.

Alkaline phosphatase activity is also considered to be a good indicator of fungal viability. Alkaline phosphatase is sequestered in the phosphatase-accumulating vacuoles in hyphae and is also found along the fungal tonoplast, but is not present in root tissue (Dickson and Smith, 1998). Other vital (and non-vital) stains are reviewed by Dickson and Smith (1998).

Laser scanning confocal microscopy

Visualization of mycorrhizal structures and quantification of their surface area and volume have recently been achieved by laser scanning confocal microscopy after staining with acid fuchsin (Dickson and Kolesik, 1999).

Chitin assay

The amount of fungal tissue present in the host tissue can be measured by chemical means. The chitin assay is based on the quantitative determination of chitin, which is present in the fungus, but not in the host. Total chitin is measured by colorimetric assay after conversion into glucosamine (Hepper, 1977; Bethlenfalvay *et al.*, 1981).

Molecular quantification

Quantification of endomycorrhizal fungi in a root system has also been achieved by molecular means using an assay based on competitive polymerase chain reaction (PCR) (Edwards *et al.*, 1997).

14.5 Identification of Mycorrhizal Fungi

Visual identification

Many ectomycorrhizas can be identified using a visual method developed and described by Agerer (1992, 1993). This method involves using both morphological features and the hyphal mantel characteristics to identify to genus or species level. Visual methods can also be used to identify some

arbuscular mycorrhizas; however, this method is of limited potential as a single mycorrhizal species may have a different morphology between host plant species.

Immunological approaches

Immunochemical detection methods are based on the reaction between antibodies and antigens. The production of antibodies is an immune system response to alien substances (the antigen). The type of antibody produced is often antigen-specific. It is this specific relationship between antibody and antigen that can be utilized as a tool in fungal identification (Göbel *et al.*, 1998; Hahn *et al.*, 1998). Antibodies, produced by exposing an animal's immune system to a specific antigen, are coupled by chemical means to molecules which can be easily detected, such as fluorescent dyes for fluorescence microscopy, enzymes for enzyme-linked immunoassays, or heavy metals for immunocytochemical analysis with an electron microscope (Harlow and Lane, 1988; Göbel *et al.*, 1998; Hahn *et al.*, 1998). Labelled antibodies introduced into a system in which the fungal symbiont is unknown will recognize and bind to their corresponding antigen (providing it is present). Schmidt *et al.* (1974) were among the first to analyse mycorrhizal fungi by immunochemical means. More recently, polyclonal antibodies have been generated with sufficient stringency to detect *Scutellospora* species in the roots of several plants (Thingstrup *et al.*, 1995). Dot immunoblots have also been used in an attempt to identify *Gigaspora* and *Acaulospora* species (Sanders *et al.*, 1992).

Isozyme analysis

Isozymes are different molecular forms of the same enzyme. They generally have the same enzymatic properties and differ only in their amino acid composition and net charge. Due to these differences in net charge, isozymes can be differentiated by their electrophoretic mobility (Rosendahl and Sen, 1994). Many isozymes are apparently species-specific in nature. Tisserant *et al.* (1998) ran protein extracts taken from root tissue colonized by five *Glomus* species on a polyacrylamide gel. The separated protein bands were stained for ten different enzymes, including alkaline phosphatase and malate dehydrogenase. Different banding patterns emerged, revealing the presence of several mycorrhizal-specific isozymes. These mycorrhizal-specific isozymes were subsequently successfully used as fungal-specific markers in plants grown in field trials.

Isozyme analysis is also increasingly used in conjunction with PCR- and restriction fragment length polymorphism (RFLP)-based molecular

approaches to identifying both endomycorrhizal (Dodd *et al.*, 1996) and ectomycorrhizal (Timonen *et al.*, 1997) fungi in root tissue.

Mycorrhizal identification by molecular means

Molecular approaches to mycorrhizal identification are becoming increasingly popular. Wyss and Bonfante (1993) extracted genomic DNA from the spores of *Glomus versiforme* and *Gigaspora margarita* isolates. Genomic fingerprints of these endomycorrhizal isolates were generated by PCR amplification with short arbitrary primers, a process known as random amplified polymorphic DNA (RAPD-PCR). The RAPD-PCR method employs an arbitrary 10 base-pair primer, which anneals to a number of complementary sites on the template DNA. The PCR products generated are separated according to their molecular weights on an agarose gel and stained with ethidium bromide. The banding pattern on the gel reflects the overall structure of the DNA molecule used as the template. If the starting material is total cell DNA, then the banding pattern represents the organization of the genome (Williams *et al.*, 1990). Wyss and Bonfante (1993) found that different mycorrhizal species generated different banding patterns. To a lesser extent, differences were also observed between different isolates of the same species.

Isolate-specific RAPD-PCR bands have been used to generate isolate-specific PCR primers by Abbas *et al.* (1996). The DNA present in bands unique to isolates of *Glomus mosseae* and *Gigaspora margarita* was purified, cloned and sequenced. Sequence analysis subsequently led to the generation of isolate-specific primer pairs, which in conjunction with PCR technology was utilized to identify these isolates in root systems. The presence of a particular isolate in a root system is identified by the presence of a PCR product band on an agarose gel.

Ribosomal DNA (rDNA) sequences have also been extensively utilized in the generation of mycorrhizal-specific PCR primers. Using PCR, RFLP, cloning and sequencing technologies, sequence differences have been highlighted in the following rDNA regions: the small subunit (SSU)/18S gene, the large subunit (LSU)/28S gene, internal transcribed spacer (ITS) regions and intergenic spacer (IGS) regions (see Egger, 1995). These differences are apparently family/species/isolate specific and have been used to generate mycorrhiza-specific PCR primers.

Simon *et al.* (1993) used single subunit (SSU)/18S gene sequences from a number of endomycorrhizal fungi to generate family-specific primers. The SSU sequences were aligned and regions apparently unique to a family were selected for the design of family-specific primers. Members of the *Acaulosporaceae*, *Gigasporaceae* and *Glomaceae* have been successfully identified using these family-specific primers in spores and root systems.

Van Tuinen *et al.* (1998) utilized the LSU/28S rDNA region to generate species/isolate-specific primers for the endomycorrhizal fungi *Glomus mosseae*, *Glomus intraradices*, *Scutellospora castanea* and *Gigaspora rosea*. Comparable studies have been carried out on endomycorrhizas by Sanders *et al.* (1995), Clapp *et al.* (1999) and Helgason *et al.* (1999).

Ectomycorrhizas have also been subject to molecular analysis. Erland (1995) studied the abundance of *Tylospora fibrillosa* ectomycorrhizas in a spruce forest in Sweden. *Tylospora fibrillosa* could be distinguished from a large number of other basidiomycetes by the banding pattern generated by PCR amplification of the rDNA ITS region followed by RFLP analysis of the PCR product. Paolocci *et al.* (1999) also utilized the rDNA ITS region to identify *Tuber* species, which were successfully characterized on host plants by PCR amplification using species-specific ITS primers. The analysis of rDNA has also been used to compare the ericoid mycorrhizas *Scytalidium vaccinii* and *Hymenoscyphus ericae* (Egger and Sigler, 1993).

Indirect methods of quantification using spores and sporocarps

The population structure of arbuscular and ectomycorrhizas can be estimated using soil spores for arbuscular mycorrhizas (Tommerup, 1994), or sporocarps (mushrooms) for ectomycorrhizas. The spores of arbuscular mycorrhizas must be isolated from the soil (Pacioni, 1994) and can be identified to genus or species level using morphological characteristics (Brundrett *et al.*, 1996). For ectomycorrhizas, sporocarps within an area can be collected, identified and their abundance estimated. However, the spore and sporocarp production is dependent upon a number of seasonal factors (temperature, moisture), and often correlates poorly with the below-ground abundance (Tommerup, 1994; Erland, 1995). Additionally, a number of important ectomycorrhizas do not form sporocarps.

14.6 Estimation of Mineral Nutrient Uptake Through Mycorrhizas

Absorption, translocation and transfer of mineral nutrients by the external hyphae of mycorrhizas have been demonstrated a number of times using radioisotopes (Tinker *et al.*, 1994). Most of the attempts at quantification of mineral nutrient uptake by mycorrhizal fungi have used techniques in which separate root and hyphal compartments have been established using fine mesh (Li *et al.*, 1991; George *et al.*, 1995; Schweiger *et al.*, 1999; Jentschke *et al.*, 2000). The size of the mesh is usually between 20 and 45

μm, which allows mycorrhizal hyphae to penetrate, but restricts growth of roots. The proportion of mineral nutrients taken up by the mycorrhizas is normally estimated by determining the decrease in mineral elements in the soil (Li *et al.*, 1991), or by using radioisotopes (Schweiger *et al.*, 1999) or stable isotopes (Jentschke *et al.*, 2000) as tracers.

Transfer of nitrogen from nitrogen-fixing plants

Techniques to measure the transfer of nitrogen from nitrogen-fixing plants to non-nitrogen-fixing plants via mycorrhizal hyphae have used compartments to prevent root contact as described above (Ikram *et al.*, 1994; Ekblad and Huss-Danell, 1995). A mesh is used to allow only mycorrhizal hyphae of the donor plant to be in contact with roots or hyphae of the receiver plants (Fig. 14.2). In some cases legumes grown in split root cultures have been used (Reeves, 1992). Nitrogen transfer is estimated using ^{15}N as a tracer. ^{15}N-labelled fertilizers are applied to the donor plants, and the amounts of fixed and transferred nitrogen is calculated using ^{15}N-dilution methods (Giller *et al.*, 1991). ^{15}N is determined by mass spectrometry.

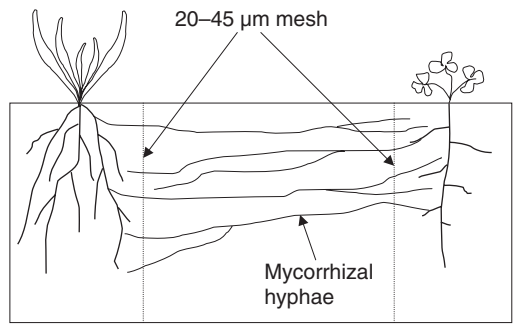


Fig. 14.2. Experimental set-up used to investigate transfer of nitrogen from nitrogen-fixing plants to non-nitrogen-fixing plants via mycorrhizal hyphae. Root and hyphal soil compartments are separated by a fine mesh of pore size 20–45 μm, which allows passage of hyphae but not roots.

Chapter 15
Rhizosphere Processes

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15.1 Synopsis

The rhizosphere can be defined as a zone of intense biological and chemical activity in the soil that surrounds the root. The rhizosphere is chemically, physically and biologically different from the bulk soil because it has been modified by roots and their associated microorganisms. Many key processes associated with soil fertility occur in this modified soil and the rhizopheres of different plants may interact. Where there are very high root densities, as in silvopastoral systems, most of the top 15–20 cm of soil may be modified by roots in this way, so that it all effectively constitutes rhizosphere soil. A comprehension of the rhizosphere is fundamental to understanding the interactions of plants with their environment and their ability to respond to stress. Different plants modify the environment around their roots in different ways and, therefore, interactions between plants may occur as a result of rhizosphere processes. The key rhizosphere processes that affect interactions between trees and soil fertility can be classified under three headings:

- chemical processes, especially those affecting nutrient capture (see Chapter 8) and metal toxicity (see Chapter 5);
- biological processes, including symbiotic associations (see Chapters 13 and 14), pathogenic activity and allelopathy (where the release of chemicals from roots of one plant affect other plants); and
- physical processes, including modification of soil structure (see Chapter 10) and its impact upon water availability (see Chapter 11).

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The vast majority of research that is done on plant interactions with soil in agroforestry focuses on gross effects of, and changes in, soil properties, rather than the detailed rhizosphere mechanisms through which these come about, but it is necessary to understand basic processes if results are to be generalized and extrapolated. Much rhizosphere research has concentrated on only a few model plant species, in very controlled conditions, and its relevance to tropical agroforestry is unclear. This means that there is considerable scope to extend rhizosphere research to determine underlying mechanisms governing interactions in economically important tree and crop associations that are used in agroforestry.

Potential importance of rhizosphere processes in agroforestry

Agroforestry systems employ two or more, often contrasting, plant species such as shade trees and herbaceous grasses in silvopastoral systems, or cereal crops and nitrogen-fixing trees in hedgerow intercropping. The premise of these systems is that the interactions between associated plants with respect to below-ground resources such as nutrients and water are complementary or facilitative rather than competitive (see Section 5.1). In some cases, one of the species may be partly chosen to stimulate above- and below-ground nutrient cycling (typically of phosphorus and nitrogen), as in the case of leguminous cover crops, legume shade trees and planted fallows. The quantification of this enhanced cycling can be achieved at a gross scale using stable isotopes and radiolabelled tracers (^{13}C, ^{15}N, ^{32}P, ^{33}P, ^{35}S; see Sections 7.3 and 8.2). However, this provides little mechanistic information. In some cases it can be shown that co-planted species have different rooting strategies that result in nutrients being taken up from different spatial zones in the soil, but in other cases rooting patterns do not provide a mechanistic basis for the complementary or competitive effects observed and the mechanisms involved can only be speculated upon (Kamara *et al.*, 1999; Lehmann *et al.*, 1999a; Ndufa *et al.*, 1999).

To fully understand why these effects occur requires an understanding of nutrient flows in the rhizosphere. For example, where enhanced phosphorus cycling is observed, the impact of both mycorrhizal and root phosphorus mobilization strategies needs to be compared in each species to identify the spatial and temporal mobilization of different soil phosphorus pools such as organic phosphorus, iron–aluminium hydroxide-fixed phosphorus and calcium-bound phosphorus (see Section 5.3). Furthermore, the plant species chosen for agroforestry systems may form different mycorrhizal associations (see Chapter 14). Trees may be either endo- or ectomycorrhizal or both, such as many species of the *Eucalyptus* genus, whereas associated crops may only be endomycorrhizal,

as is the case with maize, or may not form mycorrhizal associations at all, such as *Brassica* spp. It can be envisaged that under these circumstances the mycorrhizas will develop vastly different rhizospheres leading to contrasting patterns of nutrient capture, although experimental evidence for this form of complementary resource use is lacking. In situations where the woody plant has the same mycorrhizal association as the crop, such as a *Tithonia diversifolia* hedge next to a maize crop, then competition for soil resources may be more intense. However, as the rhizosphere of each plant species can be assumed to be unique, it is likely that resource capture by plants will always differ to some extent.

The presence of one root system, such as that of a tree, can also aid in the resource capture by a crop plant. This is evidenced by both the direct and indirect transfer of nitrogen from nitrogen-fixing plants to non-nitrogen-fixing plants (see Chapter 13). In addition, a number of other interactions may occur where trees with abundant mycorrhizas can release root exudates capable of mobilizing the large reserves of organic nitrogen or phosphorus, making it available for associated crops. While our knowledge of the rhizosphere characteristics of different plant species remains so sparse we can only speculate upon the many interactions that may occur in species associations.

Physical attributes of soil are also modified by roots in the rhizosphere and these strongly influence the growth and activity of organisms living in soil. Root exudates have been shown to significantly improve soil structure (see Chapter 10). This can occur directly in response to the root addition of polysaccharide gel (mucilage) to the rhizosphere, which helps bind aggregates together and also enhances soil–root contact (Czarnes *et al.*, 2000). Indirectly, the release of both high and low molecular weight root exudates can also enhance soil structural stability through the stimulation of the rhizosphere microbial community, which in turn produces polysaccharide gels (Traore *et al.*, 2000). As plants release different amounts of root exudates it can be expected that they influence soil physical properties in different ways. This is of relevance in an agroforestry context, where the maintenance or rehabilitation of soil structure is required and so plants may be selected specifically for their ability to enhance soil structure.

Exudation of moisture by roots into dry rhizosphere soil can also be significant in wetting soil and so facilitating nutrient uptake by the plant whose roots exude the water as well as nutrient and water uptake by other plants in the vicinity (see discussion of hydraulic lift in Section 11.1). This clearly has implications for nutrient capture from otherwise dry soil and both competitive and facilitative interactions among species with different rooting habits.

Conceptual and methodological difficulties of rhizosphere studies

Until recently, it has been difficult to achieve a mechanistic understanding of below-ground interactions between plants and the importance of rhizosphere processes in these interactions because techniques have not been available for unravelling the complexities of the processes involved. Indeed, we are still far from gaining a holistic understanding of the rhizosphere as we are only able to gain insights into specific areas for which techniques are available. Although there is conclusive evidence that the rhizosphere exists, its spatial boundaries remain difficult to define. This means that when embarking on rhizosphere research, a key step is to define an effective rhizosphere for the purposes at hand. In the case of chemicals lost from the root it is known that some may have an extensive diffusion sphere of many centimetres, whereas other highly charged compounds may only travel a few micrometres away from the root (Darrah, 1991a). Although they differ according to the chemical involved, the vast majority of chemical processes in the rhizosphere occur within a few millimetres of the root (Fig. 15.1). The exact definition of the rhizosphere and consequently the volume of soil that needs to be studied has to be relevant to the hypotheses being tested. For example, when studying allelopathic interactions in the rhizosphere, the effective radius of the rhizosphere is controlled by the diffusion of the allelopathic chemicals.

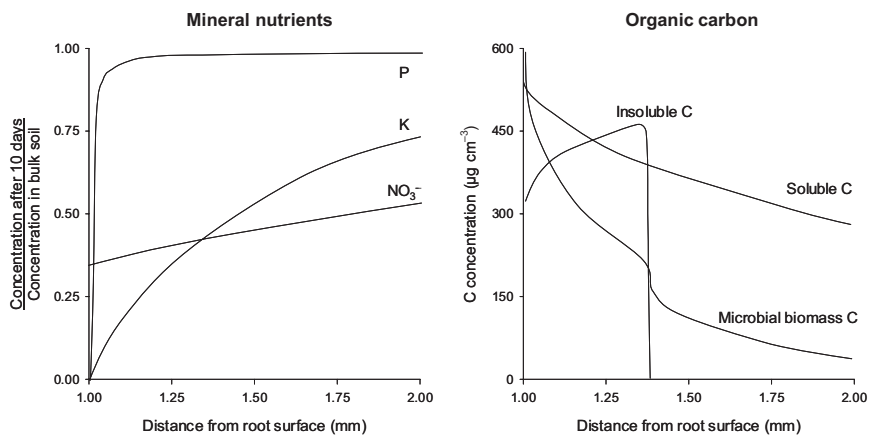


Fig. 15.1. Mineral nutrient and carbon profiles in a rhizosphere after 10 days according to simulation models based on experimentally derived nutrient uptake rates of maize and wheat. The mineral nutrient concentrations are relative to the amount present in the bulk soil at day 0 (adapted from Barber, 1995, and Darrah, 1991b).

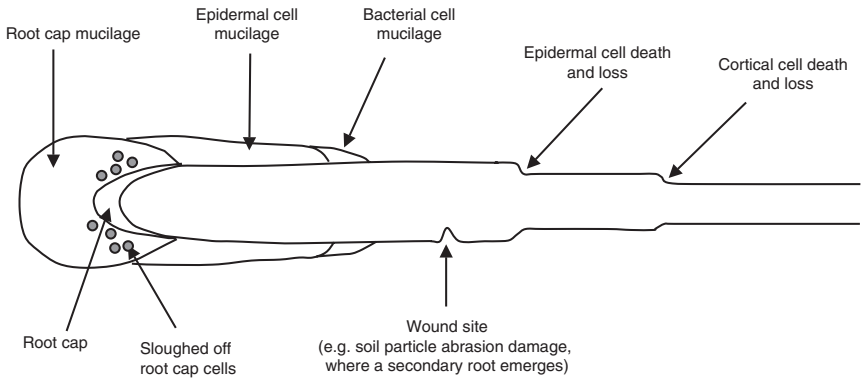

Root cap mucilage
Epidermal cell mucilage
Bacterial cell mucilage
Epidermal cell death and loss
Cortical cell death and loss
Root cap
Sloughed off root cap cells
Wound site (e.g. soil particle abrasion damage, where a secondary root emerges)

Fig. 15.2. Different functional zones of a root; root hairs are not shown.

Leaving the boundaries aside, there is also a high degree of both spatial and temporal complexity. Questions therefore arise as to whether roots should be treated as whole units or subdivided based on size, age or functional zone (Fig. 15.2). For example, root hairs (not shown in Fig. 15.2) can be usefully distinguished from the root apex. It is certainly clear from a root physiology point of view that the behaviour of different root areas is very different (Marschner, 1995). These functional root units can arise because of localized suberization/lignification, the degree of apoptosis or other forms of cell death, the different water and chemical status of roots in different soil environments or the degree of mycorrhizal infection. It is for this reason that most rhizosphere experiments are conducted in laboratory mesocosms under optimal growth conditions rather than in the field, where spatial heterogeneity makes it difficult to interpret results.

Typically, laboratory rhizosphere experimentation involves the use of seedlings and crop plants such as wheat, maize and bean. To some extent this has set a precedent so that the body of knowledge is so great for these model plants that further research tends to pursue research on these to the detriment of other species. The role of rhizosphere processes in the ecology of non-crop plants is almost unknown apart from a few isolated examples (Jones, 1998). In addition, work on trees has also been extremely limited, mainly focusing on temperate trees typically no more than a few years old due to difficulties in managing root mycorrhizal status, space limitations in climate control chambers and the short time horizon of much research (Grayston and Campbell, 1996; Grayston *et al.*, 1997).

Despite all the limitations on performing rhizosphere experiments, significant technical advances have been made in recent years which have allowed us to gain new insights into the biological interactions below ground. Although most of these techniques rely heavily on laboratory experimentation using either mesocosms (volume *c*. 50–2000 cm^3) or more

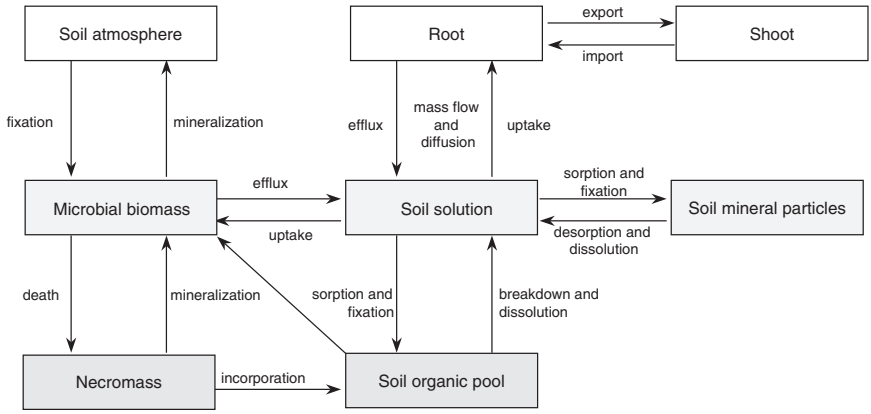


Fig. 15.3. Schematic representation of a mechanistic flux framework describing some of the possible nutrient fluxes in the rhizosphere.

often microcosms (volume *c*. 5–50 cm^3), these should not limit their potential use in agroforestry research. Although this necessitates a more reductionist approach it also reinforces the need for large multidisciplinary projects and, in the case of trees, long-term research funding. Many of the methods outlined below were first developed for looking at biological interactions in bulk soil but have since been adapted for use in small soil volumes such as the rhizosphere.

One of the major limitations to many rhizosphere studies performed in the past has been a lack of understanding of the complexity of the rhizosphere and an ignorance of key variables. For example it is common to investigate exudate release from roots without considering microbial consumption of the exudates (Matsumoto *et al.*, 1979). Where experiments are aimed at parameterizing models, it is necessary in many cases to develop a mechanistic flux framework. An illustration of this is given in Fig. 15.3. This conceptualization provides a basic decision-making tool for ensuring that all factors are being considered in order for the results to be interpretable.

15.2 Obtaining a Representative Sample of Rhizosphere Soil

Rhizosphere soil can be arbitrarily defined as the sheath of soil remaining attached to the roots after removal from the ground and gentle shaking. This offers a practical and much used approach to an almost impossible task. However, the amount of soil recovered can be highly dependent on soil and plant conditions. Soil is held to the root via a number of

Table 15.1. Comparison between root sap and soil solution nutrient concentrations illustrating the need not to contaminate rhizosphere soil samples with plant sap.

	Solute concentration (mM)		
	Root sap[a]	Soil solution[b]	Fold difference
K^+	100	0.1	1000
Ca^{2+}	8	4	2
NO_3^-	30	1.2	25
PO_4^{3-}	7	0.001	7000
SO_4^{2-}	6	0.2	30
Simple sugars	50	0.05	1000
Amino acids	10	0.1	100
Organic acids	10	0.05	200

[a]*Zea mays* root sap (includes cytoplasm and vacuolar sap).
[b]Soil solution extracted by centrifugal drainage from a free-draining Typic Haplorthod.

mechanisms including binding by water, fungal hyphae (mycorrhizal and non-mycorrhizal), adhesion with root and bacterial polysaccharides (mucilages) and adhesion via root hairs. Of these factors, soil moisture appears to largely determine the amount of soil recovered by this method, with little soil recovered when the soil becomes either too wet or too dry. There is, therefore, a critical moisture balance required for obtaining intact soil rhizosheaths and, where possible, soil moisture should be normalized across treatments. The effectiveness of the method is further reduced because soil adhesion is not usually uniform along the root length, making

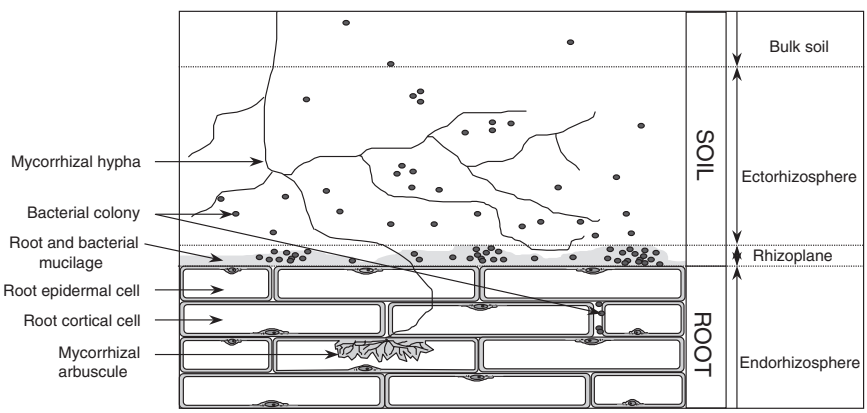


Fig. 15.4. Schematic representation of the rhizosphere zones associated with a vesicular–arbuscular mycorrhizal-infected root.

comparisons between root zones difficult. It is unusual, for example, for soil to adhere to the root apex. Once this stage has been achieved, the subsequent separation of rhizosphere soil from the root is also difficult in that the two are inextricably linked and recovery of one is impossible without some degree of contamination by the other. This is particularly important in soil or root samples that are being used for nutrient (organic and inorganic) and microbial analysis (enzymes or chemical biomass measures) as root sap solute concentrations are typically much greater than those in the soil solution (Table 15.1). Further, the most intense site of rhizosphere activity, the rhizoplane, which is the root surface, and the endorhizosphere, which is the root apoplast, are rarely recovered by this technique (Fig. 15.4).

To obtain non-contaminated and carefully controlled samples of rhizosphere soil typically requires a scaling down of experiments to a mesocosm or microcosm scale, that is, to pots or rhizotrons. Although this does not obviate performing experiments in the field, most studies performed to date have been carried out within climate-controlled chambers, enabling dismantling in the laboratory (Marschner, 1995). Briefly, roots are kept separated from rhizosphere soil through a series of nylon or polypropylene meshes. The choice of membrane is dependent on root and root hair size; however, two types of mesh are usually employed. The first is a 0.2 μm pore size membrane, which is used to maintain a sterile root compartment and a non-sterile soil compartment. This is often used to determine the effects of root exudates on soil chemical properties or microbial biomass activity changes (Meharg and Killham, 1991; Yeates and Darrah, 1991). A sterile root compartment is maintained so that exudates are not degraded by microorganisms prior to entering the soil. The second, more common approach involves the use of a 30–60 μm mesh to separate non-sterile roots from non-sterile soil. This mesh size usually does not allow penetration by root hairs (diameter 50–100 μm) but is sufficient to allow passage of microorganisms. In addition to this, successive samples away from the rhizosphere can be determined with a resolution of up to 10 μm using a microtome approach as described by Yeates and Darrah (1991) and Joner *et al.* (1995). However, the technique is not without its limitations, which include: (i) interference of water and nutrient flow by the imposition of membranes; (ii) the exclusion of root-grazing mesofauna; (iii) the exclusion of the rhizoplane and endorhizosphere; and (iv) the difficulty of spatial isolation of individual root sections required for spatial analysis. Despite these limitations, however, this type of exclusion technique has been successfully used in numerous rhizosphere investigations (Gahoonia *et al.*, 1994; Marschner, 1995; Alphei *et al.*, 1996).

15.3 Methods for Rhizosphere Soil Chemistry

Once rhizosphere and bulk (non-rhizosphere) soil samples have been obtained by the techniques outlined above, the chemical properties of each root zone can be compared by standard techniques. A typical analysis may include pH, major cations (ammonium, calcium, magnesium, potassium, sodium, aluminium), anions (nitrate, sulphate, phosphate), micronutrients (zinc, copper, iron) and dissolved organic carbon and nitrogen. Typically a variety of measures of nutrient status are performed including water or resin extraction, concentrated salt (KCl, NH_4^+-acetate), acid (HCl) or base (NaOH) extractions (Kowalenko and Yu, 1996; Trolove *et al.*, 1996; Turrion *et al.*, 1999; Zoysa *et al.*, 1999). All of these provide information on differentially available fractions of soil nutrients (see Chapter 5). Soil solution can also be removed from larger rhizosphere samples using the centrifugal drainage method described by Jones and Edwards (1993). The details of the individual chemical analysis methods can be found in Weaver *et al.* (1994), Alef and Nannipieri (1995) and Sparks *et al.* (1996).

There are now a variety of non-destructive *in situ* techniques for looking at the distribution of nutrient ions and biological activity in the rhizosphere. In the case of soil nutrient status, soil solution samplers have been designed to be able to sample gross rhizosphere solution. These are simply microversions of those used to sample bulk soil solution (see Section 7.2) and can be obtained in either ceramic, polysulphone or other organic materials (Göttlein *et al.*, 1996; Farley and Fitter, 1999). Although these do allow an estimate of rhizosphere solution chemistry to be made, the sphere of sampling is often unknown, they only operate in moist soils (<0.1 MPa) and they recover only small sample volumes. The problems of sample volume can be overcome through the use of inductively coupled plasma mass spectrometry (ICP-MS), but predicting the sampling volume is extremely difficult. Alternative more spatially specific methods involve the use of fine glass capillaries, which allow the extraction of extremely small sample volumes (10 pl), which can subsequently be analysed by capillary electrophoresis with a reasonable degree of resolution (>10 μM) (Bazzanella *et al.*, 1998). This technique is by far the most powerful; however, it requires micromanipulators, capillary pulling equipment and good dissecting microscopes and is labour intensive.

The overlaying of agar or filter papers impregnated with colorimetric indicators on to exposed roots has also been widely employed for determining rhizosphere nutrient dynamics. This technique is ideal for semiquantitative studies in which spatial dynamics are particularly important, for example when distinguishing effects of the root tip, root hair zone and root base. If the colorimetric indicators are non-rhizotoxic, then multiple exposures can be performed over time; however, in most cases only single time point measurements have been recorded. This

technique has been used widely for determining the spatial localization of pH changes in the rhizosphere and especially in response to changes in nitrogen and phosphorus nutrition. Other applications involve the study of rhizosphere phosphatase activity, root ferric reductase activity, Mn^{4+} reduction, solubilization of inorganic phosphate and the release of organic acids (Dinkelaker *et al.*, 1993a,b).

Oxygen microelectrodes have also been developed which again are capable of non-destructively determining rhizosphere O_2 concentration in a mesocosm-type system (Revsbech *et al.*, 1999). The advantages and disadvantages are discussed in Armstrong (1994). In addition, an oxygen-sensing reporter strain of *Pseudomonas fluorescens* has also been used for monitoring the distribution of low-oxygen habitats in soil (Hojberg *et al.*, 1999), as have CH_4-sensitive biosensors (Damgaard *et al.*, 1998).

15.4 Methods for Rhizosphere Biological Activity

In recent years, great advances have been made in soil microbial ecology, allowing not just the biochemical characteristics of soils to be determined, such as enzyme activity, but also the size, activity and community structure of the rhizosphere microbial population. When investigating biological activity in the rhizosphere it is advisable to adopt a multifaceted approach. Typically this will involve a basic measure of soil microbial biomass (Beck *et al.*, 1997) and microbial activity, usually basal or substrate-induced respiration (Jensen and Sorensen, 1994), alongside standard measures of key soil enzymes involved in carbon, nitrogen and phosphorus cycling such as protease, deaminase, cellulase and phosphatase activity (Alef and Nannipieri, 1995; Naseby *et al.*, 1998). Although these techniques are straightforward and can be performed easily in most laboratories, measures of soil microbial community structure often require specialist equipment. At present a number of approaches can be taken, including the use of restriction fragment length polymorphism analysis (RFLP) (Chelius and Lepo, 1999), randomly amplified polymorphic DNA analysis (RAPD) (Redecker *et al.*, 1999), fatty acid methyl ester analysis (FAMES) (Olsson and Persson, 1999; Siciliano and Germida, 1999), 16S rRNA amplification and temperature gradient gel electrophoresis polymerase chain reaction (TGGE-PCR) (Schwieger and Tebbe, 1998; Heuer *et al.*, 1999; Smit *et al.*, 1999), Biolog™ (Germida and Siciliano, 1998), enterobacterial repetitive intergenic consensus sequence PCR (ERIC-PCR) (DiGiovanni *et al.*, 1999) and phospholipid fatty acid profiling (PLFA) (Griffiths *et al.*, 1999). The most convenient of these techniques is Biolog™; however, it provides information only on culturable microorganisms and has therefore been criticized (Howard, 1997; Lawley and Bell, 1998).

Although rhizosphere biological activity can be determined in destructively sampled rhizosphere soil, this typically provides little spatial or temporal resolution. This may be especially important considering that typically only 10% of the rhizoplane is colonized and that where colonization does occur it can be highly dependent on root architecture, such as cell junctions and the endorhizosphere. However, new techniques are now available which allow microbial activity and biomass of specific species to be determined *in situ* and *in vivo*. Further, the spatial and temporal dynamics can also be determined with high accuracy. The success of these techniques, however, relies on light emission from samples and therefore experimental design is critical to their success. For this reason, experiments are typically conducted in small microcosms and can only be performed in the laboratory.

One of the main approaches has been to genetically modify target organisms with a bioluminescent luciferase (*luxAB*) gene, followed by the introduction of the tagged organism into microcosms. The amount of light produced by organisms established in the rhizosphere can subsequently be detected at a gross scale using commercial luminometers or with reasonable spatial resolution (0.05–10 cm) using a CCD-cooled camera (Deweger *et al.*, 1991; Rattray *et al.*, 1995; Prosser *et al.*, 1996). The amount of light produced by the tagged organisms can be correlated with microbial activity, allowing target organism activity as a function of time and space to be determined (Eberl *et al.*, 1997; Hagen *et al.*, 1997; Wood *et al.*, 1997). In addition, the *luxAB* gene insert can be placed into the target organism behind specific gene promoters, allowing the expression of individual microbial metabolic pathways to be assessed, such as those involved in nitrogen and phosphorus starvation (Kragelund *et al.*, 1997; Jensen *et al.*, 1998; Milcamps *et al.*, 1998).

Another similar approach has been the introduction of micro-organisms tagged with a green fluorescent protein (GFP) into rhizosphere soil. This allows detection of introduced microorganisms using standard epifluorescence and confocal microscopes. The constitutively expressed GFP can be introduced as a quasi-stable tag, allowing total population numbers to be evaluated. In contrast, the introduction of an unstable GFP tag allows the expression of specific genes and metabolic pathways to be determined, thereby providing measures of microbial activity (Gage *et al.*, 1996; Normander *et al.*, 1999; Ravnskov *et al.*, 1999; Tombolini *et al.*, 1999). In a similar fashion to that of the *luxAB* genes, the GFP cassette can also be placed behind specific promotors, allowing the study of specific metabolic pathways such as those involved in nitrogen fixation (Egener *et al.*, 1998).

15.5 Quantification of Root Carbon Loss into the Rhizosphere

The driving force for many rhizosphere processes is carbon loss from the root. Quantification of this carbon loss can be achieved in a variety of ways. The first involves whole-plant radiolabelling by continuous or pulse feeding the leaves with $^{14}CO_2$ either in the laboratory or in the field, as described by Meharg and Killham (1988). Although this approach allows total plant carbon budgets to be calculated it does not provide information on the type of carbon compounds being released and has been criticized (Meharg, 1994). To determine the spectrum of exudate loss, a number of approaches can be taken, including extraction of rhizosphere soil solution by centrifugal drainage (Ström, 1997), the collection of charged exudate compounds by ion-exchange resins (Kamh *et al.*, 1999) and the growth of plants under sterile conditions followed by collection in hydroponic or sand culture (Jones and Darrah, 1993; Hodge *et al.*, 1998). The problem associated with determining carbon loss in sterile systems is discussed in Jones and Darrah (1993). The analysis of exudate components is usually carried out by standard gas chromatography, capillary electrophoresis, high-pressure liquid chromatography and enzymatic procedures, details of which can be found in any standard biochemical methods text. In addition, sites of carbon loss can also be determined semiquantitatively with bacterial biosensors (Jaeger *et al.*, 1999).

15.6 Rhizosphere Mathematical Modelling

Mechanistically based computer simulation models are available to predict the spatial and temporal dynamics of complex rhizosphere processes (see Fig. 15.1). They incorporate parameters such as diffusion and mass flow of nutrients and water, nutrient sorption/desorption and fixation reactions, soil solution chemical equilibria and characteristics of root nutrient influx, root radius, root hairs and shoot demand. Most of the models developed have been used for predicting nutrient uptake at the root, whole plant and field level (Sadana and Claassen, 1999). The construction of these models and examples of their output are extensively described in Tinker and Nye (1999), Cushman (1984) and Barber (1995). Other types of models have been developed from this basic design into which redox reactions have been incorporated (for rice-paddy soils) or for work in model rhizosphere systems (Jones and Darrah, 1993; Kirk and Solivas, 1994; Calba *et al.*, 1999). The latest generation of models has been used to describe bacterial dynamics in the rhizosphere in response to root carbon additions (Darrah, 1991b; Scott *et al.*, 1995), whereas others have fused nutrient and bacterial

models to describe nutrient cycling involving biodegradable nutrient-mobilizing organic ligands (Geelhoed *et al.*, 1999; Kirk, 1999; Kirk *et al.*, 1999). For a recent review see Toal *et al.* (2000).

Chapter 16
Soil Macrofauna

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16.1 Synopsis

There is growing awareness that the sustainability of agricultural systems will largely depend on the adequate management of biological resources (Woomer and Swift, 1994). In the suite of factors that determine soil fertility, soil organisms are proximate regulators of nutrient availability and soil structure (Lavelle, 1997). Their importance as a resource for agriculture has been underestimated because often only constraints imposed by climate and chemical parameters of soil fertility have been considered as aspects that could be influenced by management. Since the 1980s and the new soil fertility paradigms proposed by the Tropical Soil Biology and Fertility Programme (TSBF), emphasis has been placed on the cultivation of plant species that ensure nitrogen fixation and provide organic matter and nutrients for the system while generating sufficient income to sustain farmers' livelihoods (Sanchez, 1994; Woomer and Swift, 1994). In addition to plant leaf litter and root systems, whose important roles in carbon and nutrient cycling, maintenance of soil structure and biological activity have been discussed in previous chapters of this book, soil invertebrates constitute another biological component that regulates basic soil processes such as soil aggregation, porosity and organic matter dynamics. Their management is now considered important, especially because most current land-use practices either destroy soil faunal communities or severely deplete their diversity, often with negative consequences for soil fertility (Chauvel *et al.*, 1999; Lavelle *et al.*, 1999).

In this chapter, the composition and functional structure of soil

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invertebrate communities are discussed, their effects on soil fertility are explained in broad terms, the effects of agroforestry practices on their communities are analysed, and techniques to assess their composition, abundance and activity are described.

Soil organisms and their functional domains

Soil organisms have evolved in an environment that imposes three major requirements: (i) to move in a compact environment with a loosely connected porosity; (ii) to feed on low-quality resources; and (iii) to adapt to the occasional drying or flooding of the porous space (Lavelle, 1997). A continuum of adaptive strategies based on size is observed in soils, from microorganisms to macroinvertebrates (Swift *et al.*, 1979). Soil invertebrates have been divided into micro-, meso- and macrofauna depending on the average size of the individuals. Microfauna comprises invertebrates <0.2 mm that live in the water-filled porosity of soil; mesofauna includes invertebrates 0.2–10 mm in length that live in the air-filled soil porosity and in the litter; and macrofauna comprises the largest invertebrates (>1 cm). In more general terms, a soil macrofauna taxon may be defined as an invertebrate group found within terrestrial soil samples which has more than 90% of its specimens in such samples visible to the naked eye (P. Eggleton *et al.*, unpublished). According to this definition, large individual Collembola or Acari should not be considered as macrofauna.

Microorganisms have developed the ability to digest every substrate present in soils. Unable to move, they depend on other organisms to transport them to where they can come into contact with the substrates on which they feed. Soil invertebrates have limited abilities to digest the complex organic substrates of the soil and litter, but many have developed interactions with microflora that allow them to exploit soil resources. With growing size, the relationship between microflora and fauna gradually shifts from predation to mutualisms of increasing efficiency. Excrement of invertebrates is of utmost importance in the evolution of organic matter (Martin and Marinissen, 1993), in the formation and maintenance of soil structure and, over long periods of time, in specific pedological processes referred to as zoological ripening of soils (Bal, 1982). Three major guilds of soil invertebrates, described below, may be distinguished on the basis of the relationships that they have with soil microorganisms and the kind of excrement and other biogenic structures that they produce.

- Micropredators mainly comprise microfauna that feed on bacteria, fungi and other micropredators. Microfauna do not seem to produce recognizable solid excrement and so their effect on soil organic matter

dynamics is not prolonged in structures that are stable for some time after deposition. They do, however, have a significant impact on the population dynamics of microorganisms and the release of nutrients immobilized in microbial biomass (Trofymow and Coleman, 1982; Clarholm, 1985). This process is especially developed in the rhizosphere (see Chapter 15). Predatory Acari or Collembola and even larger invertebrates (earthworms) may extend this food web over several trophic levels (Hunt, 1987; Moore *et al.*, 1993).

- Litter transformers mainly comprise mesofauna and large arthropods that normally ingest purely organic material and develop an external (exhabitational *sensu*; Lewis, 1985) mutualism with microflora based on an external rumen type of digestion (Swift *et al.*, 1979). Litter arthropods may digest part of the microbial biomass or develop mutualistic interactions in their faecal pellets. In these structures, organic resources which have been fragmented and moistened during the gut transit are actively digested by microflora. After some days of incubation, arthropods often re-ingest their pellets and absorb the assimilable organic compounds that have been released by microbial activity, and occasionally part of the microbial biomass itself (see, for example, Hassal and Rushton, 1982; Szlàvecz and Pobozsny, 1995).
- Finally, the ecosystem engineers comprise macrofauna, mainly earthworms, termites and ants, that are large enough to develop mutualistic relationships with microflora inside their proper gut. These organisms usually ingest a mixture of organic and mineral elements. Large faecal pellets (in the range of 0.1–2 cm and more) may be the component elements of macro-aggregate structures and participate prominently in the formation of stable structures through the regulation of porosity, aggregation, bulk density and surface features (Bal, 1982; Blanchart *et al.*, 1999; Decaëns *et al.*, 1999). These organisms also build large structures, such as mounds and networks of galleries and chambers, which have significant impacts on the evolution of soils over medium time scales. Ants may be considered part of this group, although the vast majority of them only use soil as a habitat and have a limited impact on soil organic matter dynamics.

Whenever conditions are suitable for their activities, macroinvertebrates, and especially earthworms and termites, become major regulators of microbial activities within their sphere of influence, referred to as the termitosphere of termites and the drilosphere of earthworms (Lavelle, 1997). Here, they also determine the abundance and activities of smaller groups of soil fauna (Dash *et al.*, 1980; Yeates, 1981; Boyer, 1998; Loranger *et al.*, 1998). These functional domains also include the rhizosphere, in which roots are the major determinants of microbial and faunal activity (see Chapter 15).

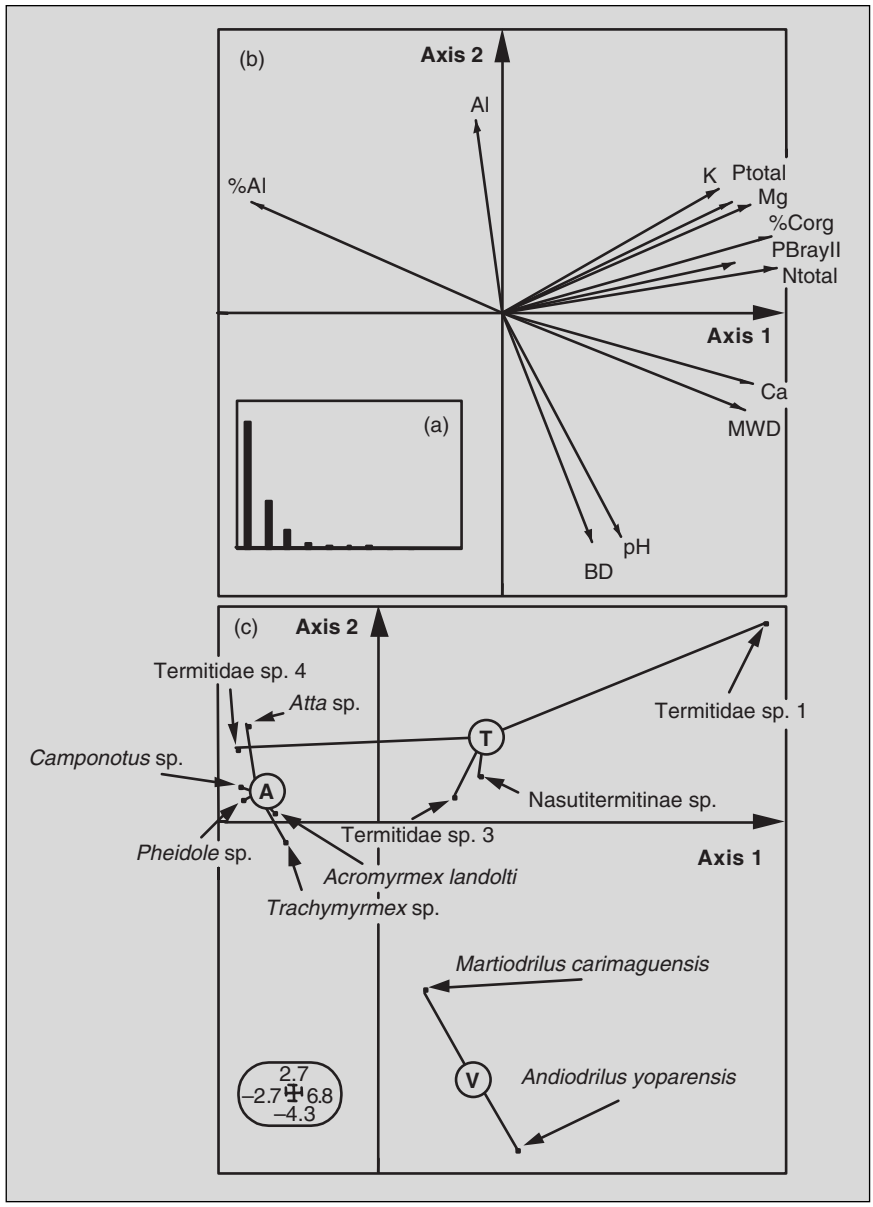
Communities of organisms in soil are thus largely interactive, with a clear hierarchical organization dominated by ecosystem engineers. This justifies putting special emphasis on the management of macroinvertebrate communities for manipulating the whole biological activity of soils that they actually regulate inside their functional domains. The sampling of macroinvertebrate communities, which comprise most ecosystem engineers and litter transformers, is therefore proposed as a suitable proxy for overall soil biological activity (see Section 16.2).

Attempts have been made to further classify macroinvertebrates into functional groups with a real functional meaning (Bouché, 1977; Grassé,

Box 16.1. Physical and chemical properties of biostructures deposited by soil invertebrate engineers at the soil surface in the eastern Llanos of Colombia.

In a natural savanna of eastern Colombia, structures deposited at the soil surface by 17 species of soil invertebrate engineers were analysed for their chemical and physical characteristics and data were treated with principal component analysis (PCA) (Fig. 16.1). The analysis separated structures into three groups corresponding to large taxonomic units (earthworms, ants and termites). Earthworm casts were characterized by high values of bulk density, pH and contents of organic matter and cations. Termite structures differed from earthworm casts in having lower pH, lower bulk density and larger diameter, which reflects a greater structural stability. Ant structures had the highest aluminium saturation and the lowest amounts of soil organic matter and cations. This study confirms that physicochemical properties of biostructures are related to the faunal taxonomic units that produce them, suggesting that these represent, to a certain extent, functional groups. An exception was the group of Termitidae sp. 4, whose sheathings were similar to ant surface nest material (Decaëns *et al.*, 2001).

Fig. 16.1. *(Opposite)* Biostructures deposited on the soil surface of the eastern Llanos of Colombia by 17 species of soil invertebrate engineers as ordinated with principal component analysis (PCA) according to their physical and chemical properties: (a) Eigen values for extracted factors (the first two axes represent 59.6 and 22.6% of total inertia, respectively); (b) correlation circles associated with the first two axes; (c) ordination of biostructures in the plane delimited by Factors 1 and 2 of the PCA (small circles are means of points representing a category of structures produced). Abbreviations: %Corg, per cent organic carbon; PBrayII, available phosphorus content (BrayII); Ntotal, total nitrogen; Ptotal, total phosphorus; Al, exchangeable aluminium; Ca, exchangeable calcium; Mg, exchangeable magnesium; K, exchangeable potassium; %Al, per cent aluminium saturation; BD, bulk density; MWD, mean weighted diameter of aggregates; V, earthworm casts; T, termites (nests of all species except for surface sheathings of Termitidae sp. 4); A, ant surface nests (reproduced with permission from Decaëns *et al.*, 2001).



1984). The effects of disturbances or changes in the environment on invertebrates indicate that large taxonomic units (such as termites and earthworms) are often fairly homogeneous groups in terms of response (*sensu*; Lavorel *et al.*, 1997) and represent a rough approximation to real functional groups, that is, groups of species that perform similar functions within ecosystems (Steneck, 2001). For example, in natural savannas of

Table 16.1. Functional categories of earthworms.[a]

Ecological category	Habitat	Diet	Pigmentation	Composting ability	Galleries	Size
Epigeic	Litter	Litter feeder	Fully pigmented	Very good	Nil or very limited	Small
Anecic	Litter and soil	Feeds on litter and soil	Anterodorsally pigmented	Fair	Long-lasting vertical	Medium to large
Endogeic (polyhumic)	Topsoil	Rich soil feeder	Nil	Poor	Horizontal, partly refilled with casts	Small
Endogeic (mesohumic)	A horizon	Bulk A_i soil feeder	Nil	Nil	Extensive horizontal galleries refilled with casts	Medium
Endogeic (Oligohumic)	A and B horizons	Deep soil feeder	Nil	Nil	Horizontal galleries refilled with casts	Large to very large

[a]After Bouché (1977) and Lavelle (1983).

Colombia, the physical and chemical properties of biostructures produced by 17 different macroinvertebrate species were clearly related to large taxonomic units, with only one exception (Decaëns *et al.*, 2001; see Box 16.1, p. 306).

Within large taxa, species can be further subdivided into functional groups of species with distinct roles in the soil. Earthworms have been classified into five different categories depending on their feeding habits and location in the soil (Table 16.1; Lavelle, 1983). These categories have different distinct effects on soil function. Epigeics are useful agents in the production of compost but poor bioturbators (i.e. organisms that mix litter and soil). Anecics create persistent vertical below-ground networks of galleries that open at the soil surface; they also produce large numbers of casts, which they deposit in soil macropores or at the soil surface. Endogeics are the most active bioturbators; they deposit most of their casts in the soil and dig horizontal below-ground galleries that they refill with their casts. They have been further divided into poly-, meso- and oligohumics with decreasing average concentrations of organic matter in the ingested soil.

Termites may be broadly classified on the basis of their feeding habits, general organization of their nests, and their location in the environment. Nests may be located in five main sites: (i) within the wood of living and dead trees and in fallen timber; (ii) subterranean nests; (iii) epigeal nests on the soil surface; (iv) arboreal nests hanging from branches or stuck to tree trunks; and (v) within the nests of other termite species (Noirot, 1970). Termites may also be separated into four groups on the basis of their feeding habits: (i) wood feeders; (ii) fungus cultivators; (iii) grass harvesters and surface litter feeders; and (iv) humivorous termites (Josens, 1983). A small number of species also regularly attack living plants. Wood feeders may be further separated into several groups, such as dry-wood, wet-wood or arboreal termites (Abe, 1987).

No specific classifications have been proposed so far for other soil and litter macroinvertebrates. A classification of ants based on their effects on bioturbation and transfers of organic matter in soil is clearly needed. Litter transformers (mainly Diplopoda, Isopoda and detritivore Coleoptera) seem to be a rather homogeneous functional group, although they may differ significantly in their response to environmental factors. Predators are mostly found in Myriapoda (Chilopoda), Insecta (Coleoptera and ants) and Arachnida.

Effects of macroinvertebrates on soil processes

Fauna effects on soil physical properties

Soil invertebrates influence soil processes directly by their digestion processes and the formation of biogenic structures. The latter effect is by

far the most important since it determines basic soil physical properties and further steps of organic matter dynamics. Soil aggregation in most humid tropical environments is determined by the activity and functional diversity of the main ecosystem engineers. In moist savannas of Côte d'Ivoire, earthworms may produce up to 1200 t of casts ha^{-1} $year^{-1}$, of which only 30 t (2.5%) are deposited on the soil surface (Lavelle, 1975). The earthworm community comprises compacting species that ingest small aggregates and produce large compact structures, the accumulation of which tends to increase the bulk density of the soil (Blanchart *et al.*, 1999), and decompacting species, which have opposite effects through the fragmentation of large aggregates into smaller ones and/or the creation of pores. The combined effects of these two broad functional categories maintain soil physical and hydraulic properties in a highly dynamic state.

Ants and termites also have strong impacts on soil physical parameters (Lobry de Bruyn and Conacher, 1990). Some Macrotermitinae may open access holes at the surface, which they use for foraging at night. The overall surface of these openings has been estimated at 2–4 m^2 ha^{-1} in a dry savanna of Kenya (Lepage, 1981). Gallery lengths of up to 7.5 km ha^{-1} have been estimated for soils associated with the mounds of *Macrotermes michaelseni* (Darlington, 1982; MacKay *et al.*, 1985; MacKay and Whitford, 1988). In the soils of Darlington's (1982) study area there was also the equivalent of 90,000 storage chambers per hectare. It can be calculated that the voids formed by termite galleries and related structures occupied approximately 0.4% of the soil volume to 20 cm depth at this site (Lavelle and Spain, 2001).

The effect of soil fauna on soil structure can be surprisingly rapid. Barros *et al.* (2001) showed that the structure of an Amazonian Oxisol could be drastically modified by changes in faunal activity in only 1 year. Soil monoliths taken from a pasture soil that had been severely compacted by an invasive earthworm were transported to a nearby forest. After 1 year of exposure to the diverse fauna of the forest, soil of the exposed monoliths had recovered the overall porosity and the percentage of macroaggregates had returned to the situation found in forest soil.

While this example demonstrates the importance of decompacting fauna species for the maintenance of soil hydraulic properties, compacting species may also play an important role through the stabilizing effect of the large and dense aggregates that they produce on soil structure which protects against mechanical stress and maintains organic matter in the interior of these dense aggregates (see below and Martin, 1991). Furthermore, an earthworm species that compacts the superficial soil horizons may increase soil porosity in the subsoil (Barros *et al.*, 2001). The dynamic equilibrium between these antagonistic activities has a strong influence on soil physical, chemical and biological properties, and illustrates the importance of the functional diversity of soil organisms for soil functioning and fertility.

Biogenic structures produced by ecosystem engineers are often rather resistant, especially after having experienced a few wetting and drying cycles. This is the case for earthworm casts that are highly unstable when fresh and soon acquire high stability after drying (Shipitalo and Protz, 1988; Blanchart *et al.*, 1993; Hindell *et al.*, 1997). Dry surface casts of *Martiodrilus carimaguensis*, a large anecic earthworm of the Colombian Llanos Orientales, can resist heavy rainfall and persist for several months before rainfall and runoff break them down into stable aggregates that form a mulch on the soil surface (Decaëns, 2000). Unless they are destroyed by another invertebrate, biogenic structures created inside the soil may persist for long periods of time after the organisms that produced them have died. Process regulation by ecosystem engineers may therefore persist long after they have disappeared, and degradation of soil properties due to their elimination by cropping practices, or any other disturbance, is often ignored because the link between the organism and the soil properties is not evident.

Soil texture in the upper soil horizons may also be modified by macroinvertebrate activities as a result of the regular deposition of finely textured biogenic structures at the surface. This process has been hypothesized to generate stone-lines found at some depth in many tropical soils (Williams, 1968; Wielemaker, 1984; Johnson, 1990). In other circumstances, fine particles deposited by fauna at the soil surface may be washed away by runoff and leave a coarse-textured surface horizon (see Chapter 17).

Fauna effects on soil organic matter dynamics

The effects of soil invertebrates on soil organic matter dynamics are disproportionate to their size and numbers. Direct mineralization of soil organic matter by earthworms was estimated at 1.2 t ha^{-1} $year^{-1}$ in a natural savanna of the Côte d'Ivoire (Lavelle, 1978). This corresponds to approximately 10% of all carbon mineralized in this soil, and the overall contribution of all soil fauna to carbon mineralization at this site probably does not exceed 20% (Lamotte, 1989). Indirect effects on organic matter dynamics are much larger, especially as they extend over several time scales. At a short time scale of days to weeks, the organic matter that has been ingested by invertebrates and egested in their faecal pellets undergoes further mineralization caused by a strong enhancement of microbial activity, labelled the 'Sleeping Beauty effect' by Lavelle (1997). At a longer time scale of months to years, organic matter occluded in compact biostructures like earthworm casts or walls of termite mounds may be physically protected from decomposition. Therefore, invertebrates that stimulate mineralization of organic matter at small scales of time and space tend to protect organic matter from further decomposition at much

larger scales. The coexistence of these two processes interacting at distinct scales may lead to misinterpretation of some experimental results. Elimination of invertebrate populations only suppresses short-term effects, whereas long-term protection of organic matter in biogenic structures will last as long as these structures are maintained. However, Blanchart *et al.* (2003) have shown that this regulation of soil organic matter dynamics via the effects on soil physical structure was only significant in soils with low clay contents and soils with inactive clay minerals of the 1:1 type, but not in soils with smectites and other 2:1 clay minerals.

Effects of agroforestry practices on soil macrofauna

Hierarchical determinants of soil function in agroforestry systems

Soil function is controlled by a suite of hierarchically organized determinants, the relative importance of which depends on the scale at which they operate (Lavelle and Spain, 2001). Agroforestry practices will affect soil function as they act on these determinants. At the top of the hierarchy are climatic factors such as moisture and temperature regimes. At the next level down are edaphic parameters with special importance accorded to the nature and abundance of clay minerals and the overall nutrient richness of soil, which is partly determined by the nature of the original bedrock. At the next level down comes the quality of organic matter produced and other parameters linked to the plant communities that are present. Macroinvertebrate communities are dependent on these determinants and constraints imposed by biogeographical factors, such as the local history and spatial distribution of land-use systems, which impinge on the maintenance of diversity and colonization of newly created cropping systems from adjacent areas. These communities, in turn, are themselves a proximate determining factor for microbial activities in the soil.

The few available data sets reflect this diversity of determinants of soil invertebrate communities (Lavelle and Pashanasi, 1989; Lavelle *et al.*, 1994; Loranger, 1999). Agroforestry systems appear to have rather specific fauna communities compared with conventional cropping systems. Trees in agroforestry systems affect living conditions of invertebrates by their shade, their deep and perennial root systems, and the quantity and quality of their litter (Tian *et al.*, 1995a; Vohland and Schroth, 1999), and by preventing soil tillage in at least part of the system.

Tillage has a significant negative impact on soil macroinvertebrates. There is a direct effect through disruption of the soil structure and the destruction of either the invertebrates themselves or the structures that they inhabit. In the longer term, a combination of microclimatic and trophic

effects prevents populations from building up rapidly since soil is left bare for some time after each tillage (House, 1985; Haines and Uren, 1990).

Soil microclimate is affected by agroforestry practices in two different ways. Soil under trees is covered with leaf litter and shaded, so that the upper centimetres of soil are protected from overheating and drying (Lal, 1986). On the other hand, the perennial nature of trees, their often high evapotranspiration and the rather deep distribution of roots may cause more intensive and deeper drying of the soil during the dry season than in areas with annual crops or other herbaceous vegetation, and this may affect soil fauna.

The quality and abundance of organic inputs is a key feature in the maintenance of diverse soil macroinvertebrate communities (Lavelle *et al.*, 2001). The provision of habitats and food for litter invertebrates favours the maintenance of specific communities of epigeic invertebrates (mainly arthropods such as Myriapoda, Coleoptera, ants or surface termites, and epigeic earthworms) and anecic invertebrates which dwell in the soil but feed, at least partly, on surface leaf litter, such as termites and earthworms. Barros *et al.* (1999) showed an effect of litter cover on soil fauna in a comparison of two different agroforestry systems and three 10-year fallows in central Amazonia. In a system with still young trees, litter cover was low and the macroinvertebrate community had a lower overall abundance and diversity and tended to live deeper in the soil than in the other sites with

Fig. 16.2. Relationships between litter dry matter, faunal density (a) and faunal biomass (b) for different sampling positions in a multistrata agroforestry system with Brazil nut trees (*Bertholletia excelsa*), cupuaçu (*Theobroma grandiflorum*), annatto (*Bixa orellana*), peach palm (*Bactris gasipaes*), a *Pueraria phaseoloides* cover crop and spontaneous grass as well as a peach palm monoculture in central Amazonia (means and s.e.; reproduced with permission from Vohland and Schroth, 1999).

older trees and more abundant litter. In the same region, Vohland and Schroth (1999) observed a close relationship between the amount of litter and the abundance of litter-dwelling macroinvertebrates. They estimated the amount of litter necessary for maintenance of an active fauna in the litter system at 3–6 t ha^{-1} (Fig. 16.2).

The amount of soil organic matter which is assimilated by an earthworm community in a natural savanna of Côte d'Ivoire has been estimated at 1.2 t ha^{-1} $year^{-1}$ (Lavelle, 1978). Based on this number, one could assume that if agroforestry practices succeeded in supplying 2 t ha^{-1} $year^{-1}$ of assimilable organic matter this should be enough to sustain macrofaunal activity at a suitable level. We do not know, however, how much litter of what quality must be applied to the soil surface to sustain a litter biomass of 3–6 t ha^{-1} as a habitat for litter-dwelling invertebrates while providing 2 t ha^{-1} $year^{-1}$ of organic matter for digestive assimilation. This question needs to be resolved for various agroforestry practices on different sites, taking the quantity and quality of available organic materials into consideration.

Assimilability of plant residues is greatly determined by their chemical composition, especially lignin, polyphenol and nitrogen contents (see Chapter 6), and this influences the composition of the faunal community. In general, the ratio of termite to earthworm abundance is dependent on the quality of the organic residues; termites tend to predominate when low-quality organic substrates are available whereas earthworms thrive better with high-quality residues. In rubber plantations of southern Côte d'Ivoire, for example, xylophagous and other termites dominate in the early phases when trunks and branches of the original forest are left on the soil surface. At later stages, 10–20 years after plantation establishment, most woody litter has been eaten up and transformed by termites, and endogeic earthworms, which presumably feed on termite excreta, become dominant (Gilot *et al.*, 1995). Tian *et al.* (1993) showed similar effects with different mulch covers in agroforestry systems in Nigeria.

In silvopastoral systems, the use of improved pastures with selected African grasses and legumes, as well as grazing, generally increases earthworm abundance and the ratio of earthworm to termite population densities (Decaëns *et al.*, 1994; B. Senapati, unpublished data). Cover crops and grass fallows may have similar impacts depending on the quality of organic matter produced. In a survey of soil macroinvertebrate communities in the Brazilian Amazon, E. Barros *et al.* (unpublished) found that mean values of the termite:earthworm density index, calculated from population densities, ranged from 0.2 in pastures to 7.9 and 8.8 in secondary forests and agroforestry systems and 20.4 and 21.4, respectively, in fallows and crops. The similarity between fallows and crops in this case indicates that the fallows were still young and so there was relatively little production of leaf and woody litter by the trees. Vohland and Schroth

(1999) found that in Amazonian agroecosystems the fleshy harvest residues of peach palm (*Bactris gasipaes*), grown for heart-of-palm production, harboured diplopods and other fauna groups sensitive to desiccation, which, in the primary forest of the region, are typically found in rotting stems of fallen trees.

Tree species effects within agroforestry systems

The distribution and nature of plants in an agroforestry system will greatly influence macroinvertebrate communities. The particular trophic and microclimatic conditions in the proximity of trees may significantly shape the invertebrate community and lead to single-tree effects on their distribution (Blair *et al.*, 1990; Boettcher and Kalisz, 1991). In a rainforest of French Guiana, endogeic earthworms were concentrated below the litter of an unidentified tree species of the *Qualea* genus but were totally absent from litter of another tree, *Dicorynia guianensis*, only 20 m away (Wardle and Lavelle, 1997). Distribution patterns of litter fauna related to the presence of individual trees have also been observed in a multistrata system with four tree crop species, a leguminous cover crop and spontaneous grasses in central Amazonia (Vohland and Schroth, 1999). Some fauna groups showed specific preferences for certain litter types. For example, earthworms were most abundant in the litter of the cover crop, *Pueraria phaseoloides*, peach palm (*Bactris gasipaes*) and annatto (*Bixa orellana*), but were absent from the hard and rather dry litter of cupuaçu (*Theobroma grandiflorum*) and Brazil nut (*Bertholletia excelsa*). Similar preferences were shown by millipedes and beetles (Fig. 16.2). In another multistrata system established after pasture in the same region, Barros *et al.* (1999) found a dominance of mesohumic endogeic earthworm species in the soil under cupuaçu and Brazil nut trees and of polyhumic earthworm species under mahogany (*Swietenia macrophylla*) and passion fruit (*Passiflora edulis*) within the same system. Presumably, this difference also reflected the low litter quality of cupuaçu and Brazil nut and, for young cupuaçu trees, the small quantity of litter produced.

At a landscape scale the distance from an agroforestry plot to areas of natural vegetation (where it still exists) and the influence of neighbouring types of land use are expected to affect the composition of fauna communities. In central Amazonia, the colonization of agroforestry plots by a large anecic glossoscolecid earthworm seemed to depend on contact with natural forest (E. Barros, unpublished). However, adequate data sets to properly test this hypothesis are lacking.

The role of biodiversity

Agroforestry systems are generally considered to have a positive effect on the conservation of biodiversity compared with simpler agricultural

systems (Fragoso and Rojas, 1994; Griffith, 2000). This is especially the case when their structure is close to that of the original ecosystem (Decaëns, 2000). Conservation of biodiversity is important both from a general ethical point of view and because it may be a precondition for the sustainable use of soils. However, this latter point is still poorly understood and limited to a few hypotheses (Hooper *et al.*, 2000; Lavelle, 2000). It is assumed that below-ground faunal diversity is influenced by the diversity of plants growing at a site and vice versa. However, the relationship is complex since a large number of interactive processes are involved. Little is known about the role of diversity in soils under field conditions beyond experimental evidence that a minimum diversity directly influences rates of basic ecosystem processes such as mineralization of carbon and nutrients (Mikola and Setala, 1998). The relationship between species richness and functional diversity seems to be rather loose since some groups, like spiders or Coleoptera, may be locally represented by more than 100 species with high apparent functional redundancy whereas termites would only have 20–40 species and earthworms approximately ten species. The latter two groups may actually occupy much wider niche spaces than non-social arthropods because of their social organization and/or ecological plasticity (Lavelle and Spain, 2001). In some cases, significant degradation of soils has been related to an imbalance between functional groups, such as invasion of an Amazonian pasture by a peregrine earthworm species, which accumulated large amounts of compact structures close to the soil surface and, in combination with depletion of decompacting species (ants and termites), led to the formation of a continuous surface crust, which impeded plant growth (see above and Chauvel *et al.*, 1999). The density of the compacting earthworm species was much lower in a small woody area (E. Barros and T. Decaëns, unpublished). The diversity of food sources and habitats characteristic of agroforestry systems may confer a lower susceptibility to such 'biodiversity accidents' than is likely in less diverse agricultural contexts. Specifically, the presence of leaf and woody litter at the soil surface is likely to stimulate the activity of decompacting species such as ants and termites, with positive effects on water infiltration (Mando and Miedema, 1997).

Conclusions and research needs

Agroforestry systems are a promising alternative to the intensive monocropping systems which were promulgated during the 40 years or so of the green revolution. Soil macroinvertebrates, especially the group referred to as ecosystem engineers, are part of the biological resources that need to be managed in these systems. Invertebrates are not only indicators of the health of the system; they are also actors participating in the maintenance of soil physical properties, short-term recycling of nutrients

and longer-term protection of nutrients and organic matter in their biostructures.

The direct management of soil invertebrate communities, by, for example, inoculation with earthworms, is difficult and costly. This makes understanding the effects of plant communities and management practices on invertebrate communities very important, so that these can be optimized through appropriate design and management of agroforestry and other land-use systems (Senapati *et al.*, 1999; Lavelle and Spain, 2001). In addition to the maintenance of a favourable microclimate and the avoidance of tillage in at least part of the system, the greatest potential of agroforestry practices to benefit soil invertebrates lies in the provision of diverse and abundant organic resources. More research, however, is needed into ways of optimizing the supply of organic resources in different agroforestry practices and under various pedoclimatic conditions, with special attention given to the effects of litters of different quality, including mixtures of different litter types, on invertebrate communities. Generating information to reconcile the partly contradictory objectives, on the one hand, to provide sufficient assimilable organic matter (through rapid litter transformation) and, on the other hand, to maintain a continuous litter layer that protects the soil and serves as a habitat, requires an integrated research approach using experimental and modelling techniques.

The composition of communities is also a relevant issue to address since the lack of functional diversity may have unexpected negative effects on soil function (Chauvel *et al.*, 1999). The composition of an invertebrate community depends on a suite of determinants that operate at different scales of time and space. Geographical constraints determine regionally or locally the pool of species present. Distance from a plot to reserves of species, such as primary or secondary forest, and the size and shape of the plot are significant primary determinants of the community composition. Inside the plot, the quality and quantity of litter produced by single plant components and the array of plant species present are of great significance. Sampling protocols and procedures for data analysis that are adapted to these sources of variability are necessary. They should integrate the spatial structure of the system at scales from landscape (watershed) to farming system and plot.

The functional classification of invertebrates is another key issue to address. Functional classification systems are still in their infancy and rarely address the real effect of invertebrates on ecosystem function. Furthermore, no comprehensive classification for all soil invertebrates really exists. Future research should seek to interpret community compositions in terms of their effect on decomposition rates, organic matter dynamics over different time scales and effects on soil physical properties.

Finally, the energy balance of faunal activities has to be considered to design appropriate management options. These should be designed to provide enough energy to sustain an adequate level of faunal activity while allowing sufficient storage of recalcitrant or physically protected organic matter for the maintenance of exchange characteristics, storage of nutrients and stabilization of soil aggregates (Lavelle *et al.*, 2001).

16.2 Sampling of Macrofauna: the TSBF Methodology

Sampling designs

A method for the collection of soil macrofauna in the field, which had originally been proposed by Lavelle (1988), has been adapted to the needs of the TSBF (Anderson and Ingram, 1993) and has recently been re-evaluated and improved within the Macrofauna Programme of the International Biodiversity Observation Year (IBOY; D. Bignell *et al.*, unpublished). The original sampling design comprised ten samples of 25 cm × 25 cm and 30 cm depth, distributed every 5 m along a transect chosen on a random basis. A problem with this design has been the strongly aggregative distribution of most invertebrate groups in the field, which results in high variance and creates autocorrelation among the sampling points. To avoid this problem, it is now recommended that the distance between sampling points is increased, whenever possible, to at least 20 m.

In agroforestry systems, two alternative protocols may be applied, which are stratified sampling or systematic sampling on a regular grid (see Section 3.4). With stratified sampling, samples of soil macrofauna are collected beneath trees of different species, if tree species effects are expected, and in the different individual components such as grass bands, crop strips or hedgerows. Ten samples per unit and certainly not fewer than five are recommended to detect significant differences between positions.

When considering communities at the scale of a farm or watershed, sampling on a regular grid is recommended. This design provides an average estimate of soil macrofauna communities in large landscape units covered by the grid and allows a stratification a posteriori by separating points on the basis of the local plant cover. Another advantage of this protocol is that the spatial structure of the plot is explicit and can be analysed with geostatistical methods (see Section 3.6). This is of great value in landscapes with land cover mosaics where movements of fauna between adjacent plots may determine the composition of communities. Spacing between sampling points will depend on the size of the area and the number of samples that can be processed with the available resources.

Hand-sorting

The sampling area is delimited, preferably with a metallic frame corresponding to the size of the sample (25 cm × 25 cm) with vertical blades of 5–10 cm on all sides that help to fix the device in position and to cut through the litter and topsoil. If litter is present, it is collected and kept in a hermetic plastic bag for further sorting. The soil sample is then isolated by cutting the soil vertically to a depth of 30 cm with a flat spade or machete. A ditch is dug on one of the four sides, and successive 10 cm strata are cut and kept in separate plastic bags for further processing.

Hand-sorting is carried out on large flat trays (40 cm × 60 cm and 5 cm deep). A handful of litter or soil at a time is spread in the tray to allow the capture of all individuals that can be seen with the naked eye. Controls of the accuracy of the method have shown that a variable proportion of individuals is found. This proportion may vary from 10% to almost 100% depending on the size, colour and mobility of the invertebrates (Lavelle, 1978; Lavelle and Kohlmann, 1984). In spite of the relatively low and variable efficiency of the sorting, the method has proven robust and highly repeatable in a wide range of tropical and temperate areas with forest, crops or grass cover (Lavelle and Pashanasi, 1989; Dangerfield, 1990; Decaëns *et al.*, 1994; Lavelle *et al.*, 1994). The results are normally used directly in multivariate analyses for the comparison of different sites or treatments. Where absolute values are important, rather than relative differences in community structure between sites, correction factors may be established for different faunal groups at the beginning of a sampling programme. This is done by sorting samples both in the field and by heat extraction (Berlese funnels) for litter and washing–sieving extraction for mineral soil (see, for example, Lavelle and Kohlmann, 1984). This approach could be especially useful in long-term studies at a single site.

It will normally take one person-day to cut and hand-sort two or three samples in the field. Since variance is always very high due to the inherently heterogeneous distribution of soil invertebrates, the recommended number of samples does not guarantee that statistically significant differences between treatments or sampling positions are obtained, even if effects are relatively large.

Identification and data storage

Although no precise guidelines on identification can be given at this stage, the invertebrates collected should be separated into a minimum list of major taxa for entry into a standardized database (Table 16.2; Fragoso *et al.*, 1999). Whenever possible, more precise identifications should be used. Families will generally yield 30–60 entries. Identifications at the species

Table 16.2. Minimum list of taxonomic units for soil macrofauna for inclusion in the MACROFAUNA database.[a]

Earthworms
Ants
Termites
Coleoptera adult
Coleoptera larvae
Total Coleoptera
Arachnida
Diplopoda
Chilopoda
Total Myriapoda
Isopoda
Dictyoptera (Blattoidea)
Hemiptera
Dermaptera
Lepidoptera larvae
Orthoptera
Gasteropoda
Other macrofauna

[a]After Fragoso *et al.* (1999).

level should be attempted if the requisite taxonomic expertise is available. General identification keys are not yet available but identification tools will be progressively released as part of the IBOY programme on macrofauna described above. These tools will be made available along with access to the MACROFAUNA database on the Internet (www.bondy.ird.fr/bondy.lest). The use of morphospecies or recognizable taxonomic units (RTUs) is recommended when identification is not possible (Williams and Gaston, 1994; Stork, 1995).

Populations of each taxon are characterized by their numbers (individuals per m^2) and biomass. Biomass is preferably expressed as fresh weight of preserved invertebrates. Expressing biomass as dry weight considerably increases the amount of work and does not increase comparability of results unless guts are emptied of their soil or litter contents.

16.3 Other Sampling Methods

Depending on the purpose of the study and the available manpower, alternative methods may be used such as the rapid biodiversity assessment (RBA) method (Oliver and Beattie, 1993; Jones and Eggleton, 2000), or

pitfall traps designed to catch the fauna that moves across the soil surface. Collection of biogenic structures deposited at the soil surface may be an acceptable surrogate to the evaluation of abundance, diversity and activity of invertebrate engineers.

Pitfall traps

Pitfall traps are pots (approximately 10 cm in diameter and 15 cm in depth) that are fitted into the soil in order to catch the vagile fauna. Ants and non-social litter arthropods (Arachnida, Coleoptera, Myriapoda) are the most commonly caught invertebrates; earthworms, termites and all truly endogeic soil fauna are less efficiently sampled or missed altogether. The following protocol was designed to sample surface arthropods in 1 km^2 square plots in European landscapes as part of the BIOASSESS programme (J. Niemela, unpublished). Sixteen sampling points are regularly distributed across the area. At each point, four traps are installed at the four corners of a 5 m × 5 m square. Traps 8 cm in diameter are partly filled with either ethylene glycol or propylene glycol. They are covered with a rain cover placed a few centimetres above the trap. The trapping period extends over a minimum of 10 weeks and traps must be checked frequently to avoid decay and loss of material. Other sampling designs are needed for studying small-scale patterns within agroforestry plots, such as stratified sampling under different tree species, or sampling shaded and unshaded or mulched and unmulched areas. Traps are particularly suitable for such purposes (Lavelle and Kohlmann, 1984; Hanne, 2001).

Pitfall traps may be made more efficient or selective by adding specific food substrates such as jam and canned tuna fish (ants), vertebrate dung or faeces (scarabeid beetles) or wood pieces (xylophagous termites). A different trap design specifically for termites consists of plastic tubes of about 5 cm diameter with lateral openings which are inserted in the soil so that the openings are below the soil surface. The tubes are covered with a lid. Pieces of wood are placed inside the tubes and are regularly controlled for the presence of termites (Su and Scheffrahn, 1996). Preliminary tests with different types of wood are recommended (Hanne, 2001).

Collection of biogenic structures

Soil ecosystem engineers produce a great variety of biostructures that they may deposit at the soil surface, hang on tree trunks or incorporate into the soil. Earthworms produce casts, middens, galleries and resting

chambers. Earthworm casts are all made of faecal material that is generally richer in fine and organic particles than the bulk soil. Termites build epigeic nests of highly variable shapes and sizes, at the soil surface and/or in the soil, on tree branches or inside rotten trunks. They also dig a wide range of galleries and chambers and deposit surface covers and runways. Termite biofabrics and deposits may be made either of faecal material or material simply separated and worked with their mandibles. Non-faecal material may be either soil or carton, which is a soft mixture of excreta, undigested plant material and inorganic elements. The texture and organic contents of termite structures may be highly variable. Ants do not comprise geophagous populations and all their constructs are made with soil that has been removed with mandibles and deposited outside the endogeic nests, most often as accumulation of loose soil aggregates. In some cases, however, ants may build rather compact epigeic earthen nests, like *Camponotus punctulatus* in subtropical South America.

Quantifying the abundance, diversity and composition of these structures may be considered as a functional substitute for the quantitative evaluation of soil macroinvertebrate communities (see Box 16.1). As such, this approach may be used to assess the impact of different agricultural practices. Sampling protocols need to be adapted to the local conditions. Large, long-lasting structures should be counted once or twice per year over large areas, whereas short-lived structures like most earthworm casts or ant deposits can be assessed by removing them from a surface and then measuring how many new structures are produced in a given period of time. This must be done in periods without rainfall, which may wash away the most fragile structures. Biostructures in the soil can be studied and quantified with soil micromorphological techniques (see Section 10.6).

16.4 Manipulative Experiments

The effect of invertebrates on soil processes can also be assessed with manipulative experiments in the field. Litterbags are widely used to assess decomposition of litter under field conditions (see Section 6.3 and Anderson and Ingram, 1993). Litter is enclosed in a mesh made of an undecomposable material and left on or in the soil under field climatic conditions and exposed to the community of decomposers. The participation of invertebrates in decomposition is classically evaluated by using different mesh sizes that permit access by invertebrates of different sizes (e.g. 0.2, 2 and 5 mm). This technique has been widely used, so that it produces data that are readily comparable with other data sets. The major drawbacks are the confinement of organisms due to the effect of the mesh, an altered microclimate in the mesh bag and an imperfect contact

with underlying litter or soil layers. For a detailed discussion of the method see Section 6.3.

Effects of soil fauna and other biological and non-biological agents influencing the soil structure, such as roots or wetting–drying cycles, can be assessed by exposing undisturbed soil monoliths in a given soil environment and monitoring changes in their physical structure and other properties (Blanchart, 1992; Barros *et al.*, 2001). The formation of large aggregates from a soil previously sieved at 2 mm under the influence of earthworms has also been studied with this method. Re-aggregation of soil by large compacting earthworms was quite fast, with the original level of aggregation being obtained after only 1 year (Blanchart, 1992).

Chapter 17
Soil Erosion

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17.1 Synopsis

There are two basic approaches to the study of erosion rates. In the first, sediment transport rates are monitored at the outlet from a catchment. Such measurements are relatively easy to make and they integrate the effects of erosion over a variety of land uses but there are problems in determining the source of the sediment within the catchment. The second approach involves measuring processes at a number of sampling sites within a catchment. The techniques vary with the processes involved. Such data are more difficult to collect but have obvious advantages in providing information about the spatial distribution of erosion rates within a catchment and the relative contribution of different land uses. The latter approach is the most applicable to agroforestry research at a field scale, seeking to investigate the effects of incorporating a tree component into a farmed environment. If both approaches are combined within the same catchment, the results of the former may serve as a test for the successful scaling up of the results of the latter.

The need to evaluate rates of soil erosion in agroforestry arises from investigations on sloping land where soil transport is a major issue. The presence of trees in hillside landscapes complicates processes that affect soil erosion so that methodologies commonly used to evaluate it require adaptation for use in agroforestry, and it is important to have a precise definition of the objectives of the research. Considerable interest in agroforestry research has focused on the potential for erosion control, and the role of trees in the redistribution of soil and associated nutrients and moisture on farmed slopes.

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Trees in agroforestry systems can affect rates of erosion in several ways: (i) they can act as a semipermeable barrier which slows the velocity of surface runoff, and hence its erosivity; (ii) they can increase infiltration by the same mechanism and also through the improvement of soil structure (see Chapter 10) and hence reduce runoff volume (which reduces nutrient losses in runoff but can increase nutrient leaching; Ramakrishnan, 1990); (iii) they can protect the soil from the impact of rainfall by production of a litter layer and/or prunings that are used as a mulch; and (iv) they can act as a sieve barrier to eroding soil and hence change the particle size composition of eroded sediments.

The presence of a tree canopy has contradictory effects on erosion. On the one hand, trees reduce the quantity of water that reaches the soil through crown interception (see Section 11.1). On the other hand, the erosivity of rainfall may increase because the size and spatial distribution of raindrops are modified during crown passage (Armstrong and Mitchell, 1988). Raindrops intercepted by tree leaves may coalesce on leaf surfaces before emerging as larger throughfall droplets, which are more erosive, beneath the tree crown. The distribution of drop sizes under trees with different leaf morphologies has been found to vary significantly, with larger leaves generally producing larger drop sizes (Calder, 2001), although drop size also depends on leaf surface characteristics and leaf inclination. The proportion of raindrops that are modified as opposed to passing through the canopy unaffected depends on crown cover and density. Tree height may also increase the kinetic energy and hence the erosivity of drops reaching the soil. Drops reach 95% of their terminal velocity in a free fall of 8 m (Young, 1997), so a further increase in height does not greatly increase their velocity when reaching the soil. Increased erosivity of throughfall over rainfall in agroforestry systems is often well understood by farmers (Thapa *et al.*, 1995) and has been well documented scientifically (Wiersum, 1984). Some trees also produce high stemflow rates, which may increase erosion under certain conditions (Wiersum, 1985). In light of this, it is unsurprising that experimental work has clearly shown that it is not the presence of the tree canopies, but rather the direct soil protection offered by a continuous litter layer that is responsible for low erosion rates in undisturbed forest (Wiersum, 1985). This has clear consequences for erosion control in agroforestry systems, where the litter layer may be disturbed during crop cultivation.

There are two principal ways of integrating trees and shrubs to control erosion in agroforestry, which differ in the relative importance of barrier as opposed to soil cover effects. These are:

- dense, mixed agroforestry systems (multistrata systems and perennial tree crop combinations); and
- spatially zoned systems (contour hedgerows and other systems of trees and shrubs in contour-aligned belts).

The former control erosion by means of a dense, surface ground cover of plants, litter or mulch, which, if maintained, is clearly effective (Wiersum, 1984; Young, 1997), though few experimental studies have been performed. Spatially zoned systems have received greater attention, and experimental results have been instructive in establishing the role of trees on slopes in increasing spatial heterogeneity in a number of soil properties (see, for example, the discussion of terrace formation below). In an agroforestry context, a fundamental question is how landscapes with trees are positioned in a continuum from pure cropland to natural forest (van Noordwijk *et al.*, 1998) and it is important that the processes elucidated in studies of trees at various densities and in various spatial arrangements are considered in their landscape context.

Approaches to measuring soil erosion in agroforestry research

Understanding the interaction of trees and slopes on the redistribution of soil and nutrients within the area being evaluated is of fundamental importance. Strong evidence has been found for a scouring effect in contour hedgerow systems on hill slopes whereby soil is lost from the upper part of the alley between hedgerows and accumulates in its lower part, leading to a transfer of surface soil properties between the two parts of the alley (Garrity, 1996). Usually, the effects of this redistribution of soil within the alleys is such that, after some time, the differences in soil properties between the upslope and the downslope side of an alley are greater than those between areas with and without hedgerows (Garrity, 1996; Agus *et al.*, 1997; Poudel *et al.*, 1999; McDonald *et al.*, 2002). The magnitude of this redistribution depends on the intensity of tillage (Garrity, 1996) and the hedgerow spacing. The choice of hedgerow spacing depends on steepness of a slope and, in turn, determines the ratio of the area of land that is between hedgerows to that which is under hedgerows, and hence the available land for cultivation. This ratio is also important because erosion control processes, such as high infiltration rates of water, occur predominantly under the hedgerow (Kiepe, 1995), which may have significant implications for nutrient losses by leaching (see Chapter 7).

The significance of the spatial variability in soil properties for experimental design was discussed by Mueller-Harvey *et al.* (1989) and highlights the crucial importance of including a sufficient number of independent experimental blocks, which control for variation in both site conditions and farmer practice, in order to produce conclusions of general applicability. If there is significant between-block variation, treatment effects will not be detected unless it is removed from the residual sum of squares (see McDonald *et al.*, 2002, for an example). Location and layout of blocks should also take into account the evidence provided by Pickup

(1985), Westoby (1987), Nortcliff *et al.* (1990), Busacca *et al.* (1993), Garrity (1996) and van Noordwijk *et al.* (1998) that on hillslopes there will be major transfers of nutrients within the slope between areas of net erosion (sources) and net deposition (sinks) depending on the topography of the slope (even fine-scale changes that are undetectable to the human eye). Mid-slope sites may undergo little or no net soil loss. A major impact of changes in land use, such as incorporation of trees, is likely to be on this rate of within-slope transfer. Farmers have learned to adjust their land-use practices to accommodate the effects of erosion by, for example, planting different crops in zones of net loss and net deposition (van Noordwijk *et al.*, 1998), so that expensive technologies that minimize such transfer by surface runoff and erosion may not actually address farmers' needs. Ramakrishnan (1990) was also concerned that, on long hillslopes, minimization of surface runoff, by increasing infiltration, may not be advantageous to overall soil fertility and agricultural productivity, because of increased nutrient losses through leaching. Therefore, the objectives of studies need to be carefully framed to distinguish net impacts of changes in land use at the individual plot scale, at the whole hillslope scale, or on the rate of discharge into rivers at the bottom of the hillslope.

McDonald *et al.* (2002) observed that, for 14 measured soil properties, variation between blocks was much greater than that between agriculture and agroforestry treatments in an experiment in the Blue Mountains of Jamaica. This indicated that, for individual farmers operating small-scale slash-and-burn agriculture and seeking to maximize fertility of their farmland, selection of more fertile sites in which to establish new farmland was a more important decision than whether or not to adopt agroforestry practices, at least over the 5-year time course monitored by the experiment. Forest restoration through secondary succession during a fallow period may be the most effective mechanism for the recovery of soil fertility in sloping lands of the humid tropics (McDonald and Healey, 2000). However, adoption of agroforestry practices may become more important:

- if shortage of farmland and restrictions on tenure prevent farmers from selecting sufficiently fertile sites for new farm establishment;
- if long periods of cultivation lead to soil fertility falling to very low levels; or
- if it is the other products and services provided by agroforestry practices that are important rather than their impact on soil fertility.

Conducting experiments over a time frame sufficient to indicate long-term trends and to adequately account for the heterogeneity of the environment is essential in this type of research.

Accumulation of soil material above the trees is commonly observed; hedgerows clearly do have a sieve-barrier effect that causes changes in soil

texture over time. The higher sand concentrations above trees may partly be because sand is more easily moved down-slope than clay, which commonly leads to higher clay contents on sloping land (Fielding and Sherchan, 1999), and also reflects the fact that the hedgerow sieves trap larger sand particles more effectively than the finer silt and clay ones. The accumulation of soil above the hedgerows progressively reduces the slope angle, resulting in the formation of terraces, and, therefore, reduces runoff generation. Higher potassium and sodium concentrations under hedgerows may indicate that transport above the soil surface is a significant mechanism in their movement, either in the eroded soil that accumulates there or in runoff water, which is likely to have much higher rates of infiltration under the hedgerows (Kiepe, 1995). Ongprasert and Turkelboom (1996) indicated that on hillslopes in northern Thailand, higher soil fertility is found under contour hedgerows than between them. Such results are influenced by the relative importance of the sieve-barrier effect, which tends to concentrate fertile topsoil under the hedgerows, the transfer of prunings to the between-hedgerow zone, which tends to redistribute the fertility (Young, 1997), and the removal of nutrients from the between-hedgerow zone with crop harvests. While trees also influence soil fertility through litterfall inputs and root uptake, the spatial distribution of these two processes is highly variable.

Studies of erosion within the context of agroforestry research must incorporate an additional spatial dimension. All farming systems operate within the socioeconomic and natural environment, but the forces of gravity acting upon transport processes on sloping land create complex patterns of spatial variability (Gardner, 1997). Simply measuring rates of soil movement will not be adequate for understanding the complexity of factors occurring when trees are introduced in hill environments.

Developing farmers' criteria for evaluating soil erosion

Increasingly, development-oriented research aims to be participatory, and collaborations between scientists and farmers have been particularly significant in the field of soil erosion. Studies of farmers' knowledge about soils, erosion and fertility are still much more common than action research which seeks ways in which farmers can address soil erosion through developing their own knowledge and actions.

Such academic studies are important starting points for recognizing the strengths of farmers' knowledge, and where it differs from scientific knowledge. Results tend to emphasize the detail of farmers' knowledge, recognizing a wide range and diversity of soils within their resource use, and the utilitarianism of their knowledge (Woodgate, 1994; Zimmerer, 1994; Carter and Murwira, 1995; Lamers and Feil, 1995). Similarly,

farmers' knowledge about soils and soil-related processes has been found to be quite consistent across farming communities, especially with similar agro-climatic conditions. A study in Nepal (P.K. Shrestha, unpublished) shows consistency in farmers' knowledge about soil physical properties and their association with soil erosion and soil fertility across three different communities in the western middle hills. Some of such knowledge, however, is patchy or specific to a location, community or even individual farmers within a community due to differences in agro-climatic conditions, cultural tradition, gender and personal experience.

Farmers' knowledge of soils and soil-related processes has also been found to be complementary to scientists' knowledge and this can help to focus further research efforts. For example, in Nepal, comparison of farmers' perceptions of the timing of soil loss and runoff with actual measured soil loss and runoff shows that they are strongly correlated, and this may provide a shortcut to research monitoring. However, in the same areas it was found that farmers do not know about leaching losses (Shrestha *et al.*, 2003), and it has been found elsewhere that farmers know less about below-ground processes, which are difficult for them to observe, than about above-ground processes (Sinclair and Walker, 1999). Such knowledge gaps represent problems that farmers are not likely to be able to address by themselves, and can help to identify topics suitable for participatory research.

There are several reasons why it is important to involve farmers in soil erosion research, either through conducting trials on their land, inviting them to observe trials on research stations, or supporting them in conducting their own research (e.g. Okali *et al.*, 1995). The benefits are as follows.

- The research will be better adapted to the environment: farmers have detailed local knowledge of what is often a highly heterogeneous environment, especially in hilly or mountainous areas where soil erosion studies are likely to be carried out. For example, studies of farmers' knowledge in Nepal showed that agroforestry systems have quite different potentials for soil conservation in different ecological situations in the middle hills. At lower elevations, trees on cropland are acceptable to farmers, whereas at higher altitudes and on north-facing slopes, farmers find the competition between trees and crops too severe (P.K. Shrestha, unpublished). This finding can help to guide appropriate technology design.
- Explanations for results may be better informed by farmers' own observations of change over many years. There are numerous documented cases where historical change known to farmers has brought about, or alleviated, the problems visible at present. Preston *et al.* (1997), for example, have shown that contemporary severe soil

erosion in Bolivia is most probably due to overgrazing in the past, and that vegetation is in fact recovering at the present time.

- If farmers are able to observe experiments, participate in the measurements or conduct their own research, they are more likely to be convinced by the results and to interpret them in the context of their livelihood systems so that the results can be incorporated into their farming methods in appropriate ways. A tradition of participatory technology development has arisen among non-governmental organizations and merged with farming systems research methods (Martin and Sherington, 1997), but these are particularly challenging in the context of soil conservation research, and new methods are still being developed (Lawrence, 1999; Lawrence *et al.*, 2000).
- Participatory approaches can lead to shared knowledge and enhanced awareness within the community, and directly to farmers identifying indicators to measure soil erosion in their own systems, and to monitor changes in soil erosion as they or others conduct research into the effectiveness of interventions.

In order for farmers to participate in such experiments, they must use methods that are accessible and meaningful to them. Attention has focused in particular on seeking indicators of soil erosion which are identified by farmers and which can be measured by them. The relatively new group of methodologies which have grown up around participatory monitoring and evaluation (Estrella *et al.*, 2000) are helpful here, and collective experience suggests that farmers' indicators are likely to be visual, observable or countable but not requiring complex measurements or recording.

Two case studies of indicator development summarized here contrast the priorities and approaches of farmers in systems where they see soil erosion as a low-priority problem (Andean foothills, Bolivia) and a high-priority problem (middle hills, Nepal). In both cases, farmers and scientists collaborated to monitor and evaluate the success of agroforestry and other interventions for controlling soil erosion on sloping cultivated land. In Bolivia, the communities involved are living in semiarid mountainous areas with road access to the city, resulting in migration and relatively low population densities in the rural communities. Because of this and the lower rainfall, free-range livestock are also an important component of the farming system. In contrast, in the research areas in Nepal, population densities are much higher, annual precipitation is much higher (but, as in the Bolivian case, strongly seasonal and erosive) and land use is much more intensive. As the following discussion shows, the farmers in Bolivia were initially not very interested in the soil erosion aspects of the trials, but recognized the value of this aspect with time.

In the Bolivian case (Lawrence *et al.*, 2000), scientists' explanations of the value of contour hedgerows based on erosion control met with scepticism among farmers, who were, however, interested in the possibility of increasing fodder production, particularly during the dry season. As the trials developed, farmers changed the criteria that they were using to assess the trials. At an early stage, when the contour hedgerows were still small, farmers focused on potential losses brought about through the trials, and highlighted the increased labour required to prepare the land for sowing when stubble cannot be burnt; risk of losing hedgerows through browsing; and compatibility with farming practices such as ploughing by oxen. However, after one growing season, participating farmers and their neighbours began to notice both soil and moisture retention around the hedgerows, and began to express interest in establishing further trials. At this stage they were invited to explicitly identify indicators to evaluate such trials, and included soil colour, soil cover, humidity and soil content of runoff in their indicators, showing a change in perception. However, increased crop and animal production were still their priorities.

In the more structured research context in the middle hills of Nepal, where farmers were specifically invited to monitor soil erosion and had more incentive to do so, given higher population densities and more intensive land use, farmers identified the following much more detailed and specific set of indicators of soil erosion (Shrestha *et al.*, 2003): changes in the number of stones exposed on the surface; exposure of the base of terrace risers; changes in the height of the terrace risers; formation of rills or gullies; changes in soil depth; exposure of crop and tree roots; change in plant vigour and health; change in crop yield; change in outward slope of terraces; turbidity of runoff water; change in soil colour; change in soil structure. By explicitly developing soil erosion indicators with farmers, the results in Nepal provide a more detailed set of measures to analyse the success of soil conservation measures, and improve the chances of farmers and scientists communicating well.

There are several lessons to be drawn from this experience. The Bolivian experience shows us that farmers' evaluation criteria are context-specific and may evolve during the course of a trial; they may also vary according to the season. The indicators adopted by farmers are visible and readily observable and, in both cases, farmers judge success according to their own direct experience, which often relates specifically to their own fields. The work in Nepal suggests that, although farmers look at what their neighbours are doing, unless they are actually working the soil and directly observing the colour, texture and runoff quality, they will have only a vague idea of the differences in soil erosion between plots, and between different agroforestry interventions.

A trial is rarely evaluated solely on the basis of its control of erosion. Farmers are interested in looking at the effect of agroforestry (or other

technologies) on their whole livelihood system, including labour availability, food security and resource flows for priority enterprises such as fodder for cattle. As Bunch (1999) has pointed out, rapid returns and economic benefits are more important to poor farmers with a short resource management horizon than soil erosion *per se* (see also Chapter 2). This last point is an important one, and not unique to the case studies described here. It is linked to a more serious point that scientists must be careful not to ignore: that, even where soil erosion appears to the outside observer to be severe, it may not be perceived as the principal problem by farmers themselves. There are plenty of case studies where soil erosion is identified as a constraint to productivity, particularly in semiarid systems, but there are others where outsiders have focused on this as a researchable constraint which can be addressed by a technological fix, whereas local residents may be aware of other priorities or sociopolitical reasons why such technological fixes will not work (Blaikie and Brookfield, 1987; Preston *et al.*, 1997). This is why soil erosion research must take place within a social research context.

The indicators that were developed with farmers in the above case studies were based on an explicit process facilitated by researchers. Farmers do not tend to consciously develop indicators, and it is sometimes the case that participatory monitoring and evaluation can place unwarranted demands on farmers, if the results are only of use to the researchers. Nevertheless, by participating in experiments which have a short-term incentive, such as fodder production, but which at the same time may reduce soil erosion, farmers may change their perceptions: those in the Bolivian case commented that they had always thought soil erosion was an unstoppable, 'natural process'. Within 2 years of beginning contour hedgerow trials on some farmers' fields, others were copying them spontaneously, not only for fodder production but also because soil erosion was reduced and, most importantly in these semiarid hillsides, because water was retained in the soil.

It is therefore important to pay close attention to the process that is used to develop indicators. The same core process was used in the two cases described here (Table 17.1). Farmers are often unfamiliar with the concept of formal evaluation using indicators, but they are constantly observing change in the environment around them, and will be able to discuss good and bad changes easily. With careful facilitation the researchers will be able to list key words that show important change and discuss a list of them with farmers. For example, in Nepal, farmers responding to trials designed and managed by scientists noted their perceptions of good and bad aspects of the trials. These included observations about labour requirements, soil deposition and changes in crop yield, and provided a starting point for developing indicators. Once the indicators have been used in explicit evaluation and comparison of

Table 17.1. The process of developing indicators with farmers.

- Situation analysis, to improve understanding of the interlinkages in farming systems and to identify farmer perceptions
- Discussions with farmers regarding individual experiences with systems changes as a result of incorporating new farming practices
- Discussions with farmers (individually or collectively) regarding their expectations of incorporating new farming practices
- Indicator development together with farmers and researchers to show farming systems changes and impacts of new technologies
- Refining of systems indicators in the field by involving more farmers
- Use of matrix diagrams based on the identified indicators to rank and score changes

interventions or plots, their utility will be much more apparent to the participating farmers.

Research priorities

Half a century of failed soil and water conservation projects in tropical developing countries demands a reconsideration of strategy (Critchley *et al.*, 1994). Recognition of this and the changing attitude of soil and water conservation programmes since colonial times has placed much greater emphasis on people's participation in all aspects of project design and implementation (McDonald and Brown, 2000). This has resulted in the adoption, in some areas at least, of a more holistic attitude towards the maintenance of land productivity (e.g. Shaxson *et al.*, 1989; Douglas, 1994; Scoones *et al.*, 1996). Much emphasis on helping farmers to improve land husbandry and less on efforts to combat erosion alone are expected to provide a more effective solution to an old problem. It has been postulated that, if farmers find it feasible and worthwhile to improve the structure, organic matter content, porosity and nutrient levels of the soil, natural fertility will be raised and soils will recuperate and runoff and erosion be diminished (Shaxson *et al.*, 1997).

It is now well recognized that the impacts of erosion are experienced differentially, and that some land managers benefit from the processes of soil erosion and runoff through redistribution of water and nutrients on their fields; for example, Stocking (1996) cites examples from Kenya and Sri Lanka, and Rakotomanana (1991) from Madagascar. Other findings suggest that rapid soil erosion may be a passing phase and that when appropriate institutional, economic and social factors are in place then higher rural population densities may be associated with investment in environmental enrichment (Tiffen *et al.*, 1994). The dynamics of change

Table 17.2. Considerations in defining research objectives.

• Who is expected to use the research results?
• Is it necessary to convince policy makers?
• How heterogeneous is the area where research is to be conducted?
• How heterogeneous is the farming community and who makes decisions about land use/who is affected by land use?
• How are people (researchers and farmers) used to interacting in the culture in question?
• How has land use changed historically – can farmers avoid wasting time addressing causes which are historical?

and the interactions between these factors demonstrate that the assumption that more people inevitably means more erosion and greater poverty is not only simplistic but also sometimes misplaced (Blaikie, 1986; Blaikie and Brookfield, 1987). Crisis narratives have developed as a result of these simplistic assumptions leading to large-scale inappropriate and largely unsuccessful policy prescriptions, particularly in sub-Saharan Africa (Tiffen *et al.*, 1994; Hoben, 1995; Rocheleau *et al.*, 1995; Roe, 1995; Benjaminsen, 1998) but also in Himalayan regions (Ives and Masserli, 1989). The emphasis in monitoring erosion in agroforestry should not be restricted to monitoring soil movement but should take a holistic approach to monitoring and appraisal, combining a variety of indicators appropriate to the objectives of the research. The most valuable approach may often be to use a combination of farmers' and scientists' criteria, but the research must be tailored in each case to certain considerations (Table 17.2).

It should be recognized that sources of error are considerable, depending on the sampling procedures, and that absolute values of soil erosion may not be meaningful. However, relative measures of soil changes, which take account of spatial patterns of relative loss and accumulation and if monitored in a participatory manner with farmers actively involved in the design and implementation of the research, can provide reasonable confidence in evaluating agroforestry innovations.

Therefore, this chapter highlights some of the most appropriate methods for agroforestry research and the special methodological considerations required when trees are involved in erosion control rather than describing standard methods of measuring erosion rates that are well covered in many useful syntheses (e.g. Hornung, 1990; Lal, 1994; Hudson, 1995; Morgan, 1995; Stocking and Murnaghan, 2001).

17.2 Quantitative Methods

Runoff plots

There is debate as to the value of using plot-based measurements in erosion research, as they isolate the plot from the upper hillside, and can interfere with lateral drainage patterns. However, they provide a realistic means of evaluating relative treatment effects in multivariate studies (Young, 1997; van Noordwijk *et al.*, 1998). Historically, they are the most commonly used technique in soil erosion research as they measure runoff and soil loss directly. Runoff plots can be inexpensive and easily constructed, and thus a sufficient number can be installed to obtain a representative sampling of the major characteristics of an area such as slope, soil type, plant cover and agricultural practice. Morgan (1995) describes their construction and monitoring. Plots are rectangular with the long axis oriented up-slope, and consist of a border, an element to concentrate runoff at the bottom end, and a collector to contain the runoff and sediment produced. Plot borders may be sheet metal, wooden planks, concrete or earth bunds. The smaller the plot, the sharper the edges need to be to minimize the edge effect. Standard runoff plots are 22 m long and 1.8 m wide, but plots containing trees should be much larger, not less than 40 m × 10 m (Young, 1997) to counter the problems of tree roots spreading from one treatment plot into other adjacent plots (see Section 3.2). Collectors are generally a flattened funnel with a hinged cover to facilitate cleaning. The sheet metal floor of the element is installed at least 5 cm below the surface, with a strip of sheet metal on the up-slope side flush with the soil surface to provide a sharp boundary at the plot end, which empties into collecting drums with a system of divisors to cope with large volumes of runoff. The soil loss is measured by stirring the tank, taking a sample of suspended sediment, filtering and drying, and then calculating the total loss from knowledge of the tank volume. Since most runoff plots in developing countries are simple, low-cost installations, there are many sources of error depending on the sampling procedure. Zöbisch *et al.* (1996) compared the manual sampling accuracy of field staff in charge of erosion plots in Kenya. The measurements displayed acceptable accuracy for measurement of runoff volumes, but the accuracy for soil loss measurements varied considerably, with an average sampling error of 41.3%. Stocking (1996) attributes this error to inadequate mixing of the solution in the tank. Even with vigorous stirring, it is difficult to get a representative sample as clays are over- and sands under-represented in the sludge samples. For large tanks, a good approach is to empty them successively, e.g. with a bucket, and collect a suspension sample at regular intervals, e.g. from each fifth or tenth bucket, depending on the total volume. After emptying the tank, the sandy sediment on the bottom of the

tank is collected as a separate sample. After drying all samples, the quantity of eroded soil is calculated by multiplying the average concentration of suspended soil per unit volume of water from the suspension samples with the total water volume in the tank and adding to this the bottom sediment (T. Morshäuser, unpublished).

Runoff plots have been used in several agroforestry studies (e.g. Hurni and Nuntapong, 1983; Maass *et al.*, 1988; Lal, 1989; Agustin and Nortcliff, 1994; Alegre and Rao, 1996) and modifications have included use of Gerlach troughs (Maass *et al.*, 1988; McDonald *et al.*, 2002) or catchpits (Hellin and Larrea, 1997). These are described below.

Difficulties arise in scaling up results from such plots to farmers' fields, hillsides or catchments, so, for a better understanding of erosion as a multiscale process, other indicators of processes within standard plots should also be observed (see subsequent sections). This is particularly important as agroforestry research evolves from a focus on field-scale technologies to a more complete consideration of landscape-scale processes and constraints involving a farmer-led process of technology development (van Noordwijk *et al.*, 1998).

Back-sloping terraces: a special case

Runoff plots were developed for uniform slopes with gentle gradients in the USA (Wischmeier and Smith, 1978). Hillside terraces are far smaller than fields, and a standard runoff plot will therefore encompass upwards of three terraces. As yet, there is no standard means of quantifying runoff and erosion losses from such terrain (Hudson, 1992). With the back-sloping terraces common throughout South-east Asia, bounded plots will dissect lateral drainage paths (toe-drains), and the collecting drums may effectively collect only from the lowest terrace, giving artificially low values (Bruijnzeel and Critchley, 1996). Bruijnzeel and Critchley (1996) have suggested the concept of a natural boundary erosion plot based on a single-terrace unit (bed and up-slope riser) with its actual boundaries (Fig. 17.1). The toe-drain is located at the foot of the riser, so runoff and eroded material are channelled out of the individual terrace unit and can be conveniently monitored at the outlet. This will also collect uncontrolled surface run-on or subsurface water input from upper slopes (Gardner *et al.*, 2000), so such measurement devices should be installed on several terraces in different positions within the slope to obtain an overall picture. Bruijnzeel and Critchley (1996) suggest another, specific metholodogy to determine the source of the eroded material. This involves installing a set of removable terrace riser troughs, which collect runoff, sediment washed down from the riser face and, presumably, any subsurface water flow emerging from the riser face (Fig. 17.1).

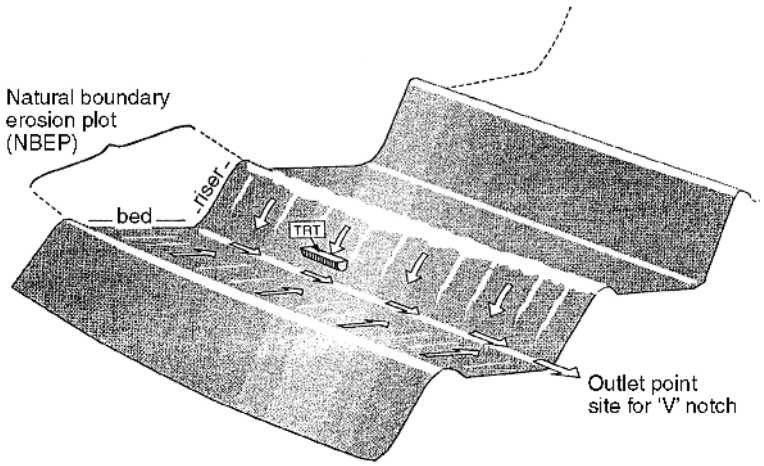


Fig. 17.1. Natural boundary erosion plot with one terrace riser trough (TRT) in place (reproduced with permission from Bruijnzeel and Critchley, 1996).

This area of research will become more relevant to agroforestry research if the objective is to control riser erosion and increase the productivity of terraced systems, e.g. by inclusion of perennial fodder grasses and legumes, with stabilizing fodder trees planted on the top of the riser (Shrestha *et al.*, 2003).

Gerlach troughs

This method is described in detail by Morgan (1995). Gerlach troughs are simple metal gutters, usually 0.5 m long and 0.1 m broad, closed at the sides and fitted with a removable lid. An outlet pipe runs from the base of the gutter into a covered collecting vessel (Fig. 17.2). They are normally sited at different slope lengths and the collecting area assumed to be equal to the width of the trough times the slope length (Fig. 17.2). On non-uniform slopes, the gutters should be sited to collect down-slope and not lateral drainage. The lack of plot boundaries eliminates any edge effects. These gutters can therefore be used without runoff plots (e.g. McGregor, 1988) to investigate the interaction of soil status and soil erosion with land use. They provide a relatively efficient means of covering a range of conditions within a site. They have been used to collect runoff and eroded soil from small bounded subplots nested within a treatment plot (e.g. Ross *et al.*, 1990) but, clearly, this is not an applicable approach in plots containing trees. Small plots will allow investigations into infiltration and the effects of rain splash but are too short for studies of overland flow except as a transporting agent for splashed particles (Morgan, 1995).

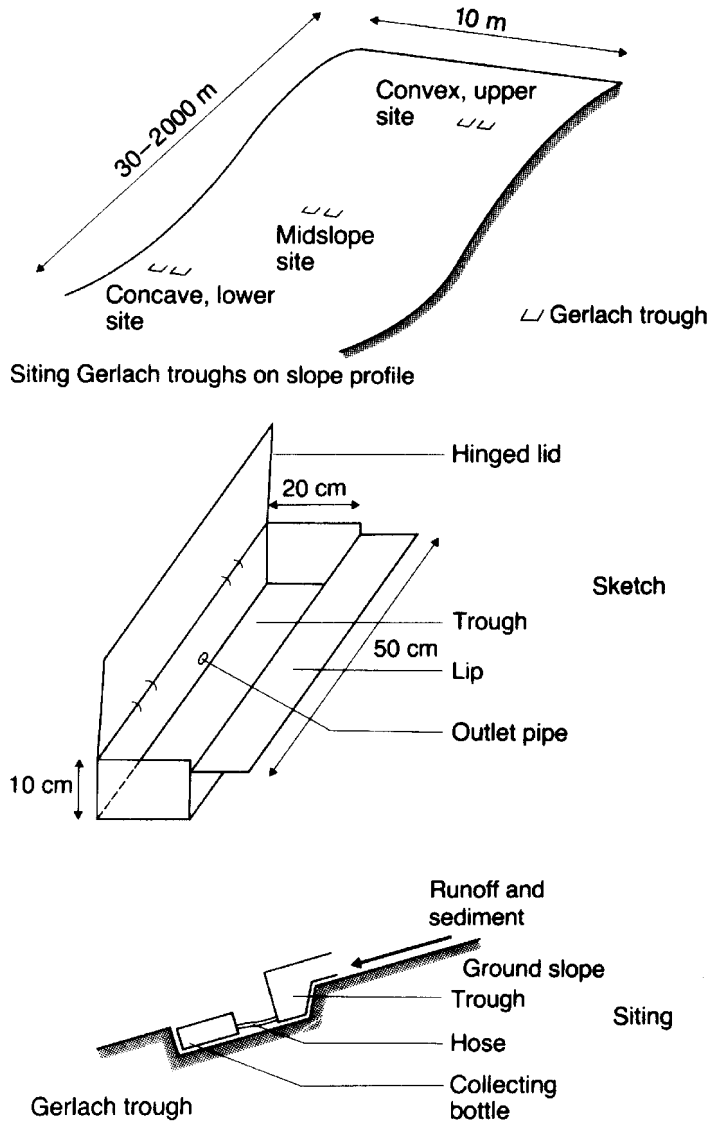


Fig. 17.2. Gerlach troughs (reproduced with permission from Morgan, 1995).

Catchpits

Catchpits are collecting pits that are dug into the ground at the bottom of the study area and lined with plastic or concrete to render them impermeable and that fill with runoff water and eroded soil. Catchpits can be effective in measuring amounts of sediment lost and runoff volumes if

the reservoir is of sufficient capacity, although smaller pits that only catch an unknown proportion of the sediment can still be used to obtain comparative information. Pits of around 10 m^3 have been used in Thailand (Hudson, 1995). Obviously, durable plastic is required that will not be holed by soil fauna or degraded by sunlight. Hellin and Larrea (1997) used catchpits lined with plastic that had been punctured with small holes so that runoff water slowly dissipated and the sediment load was retained until end-of-season measurement. They found soil loss to be within the same range as that recorded by barrels below similar plots. Catchpits have the added advantage of providing a visual display for farmers of the effectiveness, or not, of the practice under test.

Stakes and pins

Repeated measurement of the height of the ground surface at stakes or erosion pins has been used to estimate erosion. Instrumentation consists of a long nail or stake and a large washer. Stakes should be made from metal or another resistant material as wooden stakes are rapidly attacked by termites under tropical conditions. At the time of installation, the nail is driven into the soil, with the washer lying on the soil surface. The distance from the head of the nail to the top of the washer is then measured (Fig. 17.3a). Erosion moves material from around and beneath the washer. Remeasurement of the distance between the top of the nail and the top of the washer provides a measure of the rate of erosion in the intervening period (Fig. 17.3b). If the washer has protected the soil from raindrop impact, so that it now stands on a pedestal, the pedestal must be removed before measurement, so that the washer lies at the general ground surface. The advantage of using the washer is that it gives a firm surface from which to measure. The washer can also be removed between two measurements. Marking the pins with bright paint aids relocation. Such measures are cheap and easy to install over a wide range of conditions. A disadvantage of the method is that it cannot be used on tilled soil because of the change of soil surface caused by tillage and the subsequent resettling of the soil.

Instead of measuring soil removal by erosion, pins can also be used to measure soil deposition. In a sloping area in central Togo, G. Schroth (unpublished) used the method to determine patterns of sediment deposition in strips of trees and hedgerows which had been planted on the contours to collect soil lost from adjacent tilled crop fields where the pin method could not be used. Pins could be a cost-efficient method to determine patterns of net erosion and deposition over entire slopes (see Section 17.1).

Instead of measuring directly around the pins, a cable or other rigid device can be placed between two pins or stakes. With this improvement

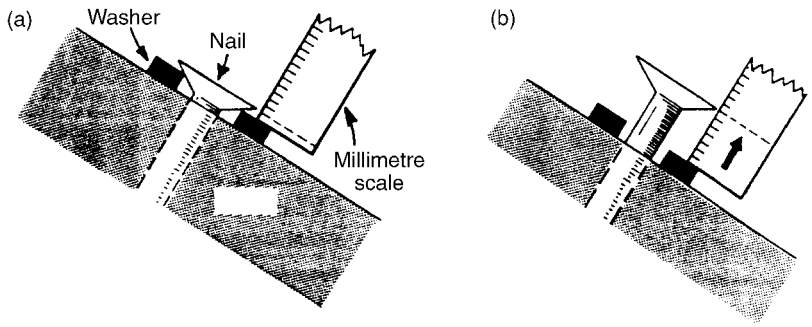


Fig. 17.3. Measurement of erosion and deposition at stakes: (a) installation; (b) remeasurement (reproduced with permission from Dunne, 1977).

of the pin method, Sims *et al.* (1999) evaluated the effects of barriers on soil retention with the help of permanent benchmarks above and below them. Measurements were made to the soil surface from a taut cable tied to the benchmarks, which indicated the accumulation or loss of soil before and after each rainy season. This was combined with annual measurements of slope of the interbarrier soil to indicate changes in microtopography.

17.3 Qualitative Methods

Tree root exposure or soil pedestals

It is sometimes possible to reconstruct the recent erosion history of an area from truncations of soil profiles, from the height of residual soil pedestals, or from the exposure of tree roots. Normal spatial variations of soil profile depth with slope gradient must be taken into consideration. If soil erosion is rapid following the destruction of vegetative cover, remnants of the former surface may be left. A ruler, tape or frame laid across the former surface can be used as a reference from which to measure the average depth of erosion (Fig. 17.4a). The exposure of tree roots can also be measured (Fig. 17.4b). However, some trees, even on undisturbed sites, grow with part of their roots above the ground surface. So, before such measurements are made, trees of the same species in neighbouring areas should be investigated.

Changes in soil microtopography

Evidence of the breakdown of the surface soil structure and its subsequent transport across the soil surface can be seen in the change in microtopo-

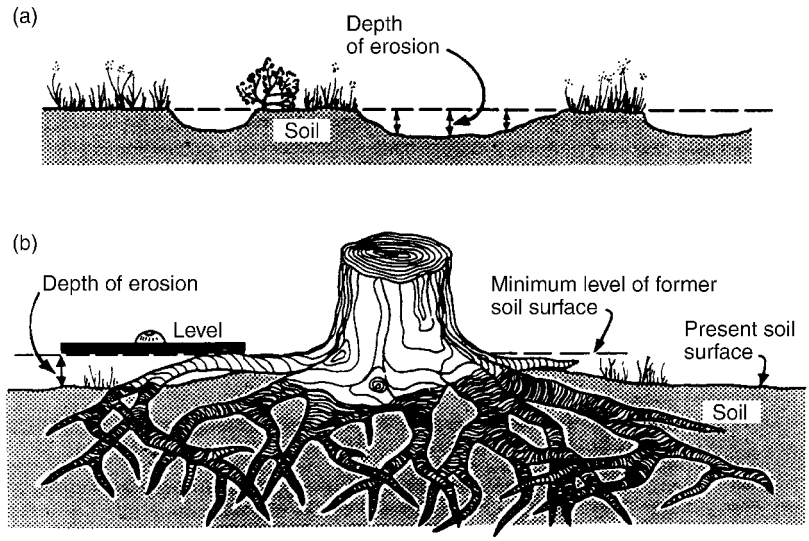


Fig. 17.4. (a) Measurement of erosion between vegetated remnants of former soil surface; (b) measurement of erosion around tree roots (reproduced with permission from Dunne, 1977).

graphical features of the soil surface. These include the breakdown of clods formed by ploughing; the filling of ploughed depressions by soil loosened from the clods and from the rest of the soil surface; and indicators that water has flowed across the soil, such as flowlines and rills. Ranking schemes can be developed to undertake series of structured field observations of the evidence for soil loss provided by these features (Bergsma, 1992). Stocking and Murnaghan (2001) suggest developing a context-specific ranking scheme which, if used consistently, can be a good way of combining indicators to give a more comprehensive view of land degradation. For example, to assess rill erosion, a scale of 0–3 indicates the development from no rills present to the presence of deep rills (up to 300 mm depth) and/or rills affecting more than 25% of the surface area. The FAO (1976) advocates wider ranking for the same parameter with a scale from 0 to 14, which describes a range from no visual evidence of rills, to rills present 7.5–15 cm deep at intervals less than 1.5 m. Utilization of numerically wider ranges has the added advantage of being more sensitive to the determination of statistical significance. The FAO (1976) and Bergsma (1992) apply such rankings to a range of topographical features including flow patterns, gullies, surface litter accumulation and depressions. Sources of error can include classification of the features, a low frequency of observations, and mislocating the same location for repeated recordings (Bergsma, 1992). Special attention should be given

to signs of runoff and transport of eroded soil at the field boundaries, where soil is effectively lost from the field, i.e. on the down-slope side in all fields and on the lateral sides in contour-ridged and terraced fields.

Stable isotopes

Caesium-137 is a by-product of the nuclear bomb testing that occurred in the 1950s and 1960s. It has a relatively long half-life of 30.17 years and does not occur naturally in the environment, so can be used as an indicator of disturbance if comparison is made between undisturbed areas and those subject to perturbation by erosion. The technique has been applied successfully to terraced areas in China and Nepal (Quine *et al.*, 1992; Zhang *et al.*, 1994; Gardner *et al.*, 2000). Gardner *et al.* (2000) noted very high spatial variability, with the worst cases indicating losses roughly equivalent to 40–50% of the top 10 cm of soil over the 40-year period. They concluded that there was no systematic spatial pattern of net loss or accumulation down-slope associated with natural convexities or concavities in the slope topography, and that the differences were more likely to reflect localized pathways of preferential drainage and water movement. Their results highlight the processes of redistribution of soil on a hillslope scale, and indicate the usefulness of this technique as a relative tool for qualitative assessment of soil erosion to indicate patterns of spatial loss and accumulation. The extension of the technique to provide quantitative estimates of loss remains to be developed (Walling and Quine, 1990). Estimates of soil loss using caesium-137 should be compared with other means since they may be subject to errors arising from uneven mixing of the cultivated layer and preferential adsorption of the isotope on to clay particles during the processes of erosion, transport and deposition (Morgan, 1995).

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